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Phylogeny of xerophilic aspergilli (subgenus *Aspergillus*) and taxonomic revision of section *Restricti*

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Abstract: *Aspergillus* section *Restricti* together with sister section *Aspergillus* (formerly *Eurotium*) comprises xerophilic species, that are able to grow on substrates with low water activity and in extreme environments. We adressed the monophyly of both sections within subgenus *Aspergillus* and applied a multidisciplinary approach for definition of species boundaries in sect. *Restricti*. The monophyly of sections *Aspergillus* and *Restricti* was tested on a set of 102 isolates comprising all currently accepted species and was strongly supported by Maximum likelihood (ML) and Bayesian inferrence (BI) analysis based on β-tubulin (*benA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) loci. More than 300 strains belonging to sect. *Restricti* from various isolation sources and four continents were characterized by DNA sequencing, and 193 isolates were selected for phylogenetic analyses and phenotypic studies. Species delimitation methods based on multispecies coalescent model were employed on DNA sequences from four loci, i.e., ID region of rDNA (ITS + 28S), *CaM*, *benA* and *RPB2*, and supported recognition of 21 species, including 14 new. All these species were also strongly supported in ML and BI analyses. All recognised species can be reliably identified by all four examined genetic loci. Phenotype analysis was performed to support the delimitation of new species and includes colony characteristics on seven cultivation media incubated at several temperatures, growth on an osmotic gradient (six media with NaCl concentration from 0 to 25 %) and analysis of morphology including scanning electron microscopy. The micromorphology of conidial heads, vesicle dimensions, temperature profiles and growth parameters in osmotic gradient were useful criteria for species identification. The vast majority of species in sect. *Restricti* produce asperglaucide, asperphenamate or both in contrast to species in sect. *Aspergillus*. Mycophenolic acid was detected for the first time in at least six members of the section. The ascomata of *A. halophilicus* do not contain auroglaucin, epiheveadride or flavoglaucin which are common in sect. *Aspergillus*, but shares the echinulins with sect. *Aspergillus*.

Key words: *Aspergillus restrictus*, *Aspergillus penicillioides*, *Eurotium*, food spoilage, indoor fungi, linear discriminant analysis, multigene phylogeny, multispecies coalescent model, sick building syndrome, xerophilic fungi.

Taxonomic novelties: ***Aspergillus canadensis*** Visagie, Yilmaz, F. Sklenar & Seifert, ***Aspergillus clavatophorus*** F. Sklenar, S.W. Peterson & Hubka, ***Aspergillus destruens*** Zalar, F. Sklenar, S.W. Peterson & Hubka, ***Aspergillus domesticus*** F. Sklenar, Houbraken, Zalar & Hubka, ***Aspergillus glabripes*** F. Sklenar, Ž. Jurjević & Hubka, ***Aspergillus hordei*** F. Sklenar, S.W. Peterson & Hubka, ***Aspergillus infrequens*** F. Sklenar, S.W. Peterson & Hubka, ***Aspergillus magnivesiculatus*** F. Sklenar, Zalar, Ž. Jurjević & Hubka, ***Aspergillus pachycaulis*** F. Sklenar, S.W. Peterson, Ž. Jurjević & Hubka, ***Aspergillus pseudogracilis*** F. Sklenar, Ž. Jurjević & Hubka, ***Aspergillus reticulatus*** F. Sklenar, Ž. Jurjević, S.W. Peterson & Hubka, ***Aspergillus salinicola*** Zalar, F. Sklenar, Visagie & Hubka, ***Aspergillus tardicrescens*** F. Sklenar, Houbraken, Zalar, & Hubka, ***Aspergillus villosus*** F. Sklenar, S.W. Peterson & Hubka.

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INTRODUCTION

Aspergillus section *Restricti* species occurs in environments with low water activity (a_w). They are commonly found on building materials, in house dust (Kaarakainen *et al.* 2009, Visagie *et al.* 2014) and dried, salted or high sugar content foods (Pitt & Hocking 2009, Frasz & Miller 2015). Much attention is being paid to the indoor air quality (Kasuga 2012, Flannigan *et al.* 2016) and these fungi are repeatedly reported to be present in this environment (Samson *et al.* 2002, Meklin *et al.* 2004, Meklin *et al.* 2007), where they are considered a potential agent responsible for sick building syndrome, respiratory problems and allergies (Terr 2009, Saijo *et al.* 2011, Abe 2012). Species may cause post-harvest rot in improperly dried commodities such as maize or wheat (Christensen & Kaufmann 1965), cotton goods are susceptible to *A. restrictus* rot (Smith 1931), while *A. vitricola* can even damage optical instruments (Ohtsuki 1962). Recently, fatal disseminated aspergillosis in an infant was proved to be caused by *A. penicillioides* (Gupta *et al.* 2016) and *A. conicus* was detected as the causal agent of an intraocular infection (Smith *et al.* 2013). While there are reports in the literature with infection cases from *A. restrictus*-like fungi, the patient nearly always has a documented underlying disease state, suggesting opportunistic infections (de Hoog *et al.* 2009).

Secondary metabolite production in these species has not been studied extensively. According to Micheluz *et al.* (2016), the

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homothallic species *A. halophilicus* produces many metabolites (chaetoviridin A, deoxybrevianamid E, pseurotin A, pseurotin D, rugulusovin, stachybotryamide and tryprostatin B) compared to anamorphic species producing a much narrower spectrum of substances with only asperglaucide detected in *A. penicillioides* and some unspecified metabolites in *A. vitricola* (Micheluz *et al.* 2016). There are no known mycotoxins produced by members of sect. *Restricti* and they do not pose a direct threat to consumers, but they cause significant losses for food and agricultural industries (Deschuyffeleer *et al.* 2015). More worrying is their potential for creating more favourable conditions for less xerophilic fungal species that may produce hazardous mycotoxins. They do this by producing metabolic water, thereby increasing water activity of the substrate. From a biotechnological perspective, polyextremophilic α-amylase produced by *A. penicillioides* has significant potential for use as a detergent (Ali *et al.* 2015).

The first species described from this section was *A. penicillioides* observed from Argentinian sugar cane (Spegazzini 1896). *Aspergillus caesiellus* was subsequently described from air in Tokyo (Saito 1904), *A. gracilis* from a *Monilinia fructigena* fruiting body and at the time thought to resemble *A. fumigatus* (Bainier 1907). Blochwitz described *A. conicus* from chalky soil (Dale 1914); Smith (1931) described *A. restrictus* causing degradation of cotton in the manufacturing proces; and finally, *A. vitricola* was described from binocular lens (Ohtsuki 1962). The taxonomy of *Aspergillus* was advanced greatly by the designation of type specimens and ex-type cultures by Samson & Gams (1985). Types for all aforementioned species in sect. *Restricti* were summarised or newly designated with the exception of *A. vitricola* (was not accepted by the authors). Pitt & Samson (1990) reduced the section to three species on the basis of physiology and morphology. Peterson (2000) used a phylogenetic analysis of LSU rDNA (28S) sequences to provide genetic evidence that there are seven species in the section, including *A. halophilicus*, which was described as *Eurotium halophilicum* from corn seeds by Christensen *et al.* (1959). Peterson (2008) used multilocus DNA sequence data, phylogenetic and concordance analysis to produce a statistically supported analysis of sect. *Restricti* containing seven species.

The morphology of sect. *Restricti* species is very simple and the number of taxonomically relevant morphological characters is low. Correct identification based solely on morphology is therefore challenging if not impossible and sequence comparisons represent the best method for fast and robust identifications. Phylogenetic analysis based on multiple loci has become an indispensable part of taxonomic studies. A polyphasic approach to species delimitation is currently standard in *Aspergillus* (Samson & Varga 2009, Samson *et al.* 2014) with the phylogenetic component usually relying on the genealogical concordance phylogenetic species recognition (GCPSR) approach proposed by Taylor *et al.* (2000).

New and advanced multi-locus methods for species delimitation were introduced recently (Fujita *et al.* 2012, Tang *et al.* 2014, Fontaneto *et al.* 2015). The majority of new approaches and associated software is based on coalescent theory and multispecies coalescent model (Flot 2015). Simultaneously because of the possible incongruence between gene trees, the focus is also shifting from gene tree to species tree inference using methods, that take into account incomplete lineage sorting (ILS), the most common cause of locus incongruence (Edwards 2009). We followed the suggestion of Carstens *et al.* (2013) that unites the two mentioned tasks into one analysis, i.e., species delimitation and species tree estimation. Firstly, species are delimited from a set of individuals by several species delimitation methods, possibly based on different approaches (based on trees, genetic distances, haplowebs, etc.). Some methods or some loci may be more prone to over delimitation (i.e., the method splits the dataset into more potential species compared with other methods or loci) of species than others, so it is recommended to compare more methods with as much loci as possible (Carstens *et al.* 2013). The species tree is subsequently inferred with individuals assigned into putative species based on the results of species delimitation. These methods are currently being applied to many different groups of organisms (Flot 2015) but infrequently in fungi. In this study, we applied them for the first time to delimit species boundaries in *Aspergillus*.

During identifications of isolates of sect. *Restricti* from various substrates and locations, we encountered many isolates that could not credibly be placed in the seven species accepted by Peterson (2008). In order to substantiate the initial finding we assembled a set of 193 isolates from sect. *Restricti* including type isolates, conducted DNA sequencing of four genetic loci, coalescence analysis, physiological testing (temperature and a_w gradients), micro- and macromorphology and SEM (scanning electron microscopy) in order to describe the biodiversity found in this section. Additionally, the monophyly of section *Restricti* within subgenus *Aspergillus* was addressed.

MATERIALS AND METHODS

Fungal strains

Strains used in this study were obtained from and deposited into various culture collections: 1) ATCC, American Type culture collection, Manassas, Virginia, USA (https://www.lgcstandards-atcc.org/en/Products/ATCC_Genuine_Cultures.aspx); 2) BCCM/IHEM, Biomedical Fungi and Yeasts Collection, Scientific Institute of Public Health, Brussels, Belgium (http://bccm.belspo.be/about-us/bccm-ihem); 3) CCF, Culture Collection of Fungi, Charles University, Prague, Czech Republic (https://www.natur.cuni.cz/biology/botany/structure/culture-collection-of-fungi-ccf); 4) CBS, culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (http://www.westerdijkinstitute.nl/Collections); 5) DAOMC, Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; 6) DTO, working collection of the Applied and Industrial Mycology department housed at the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; 7) EMSL, EMSL Analytical Inc., New Jersey, USA (http://www.emsl.com); 8) EXF, Culture Collection of Extremophilic Fungi, University of Ljubljana, Slovenia (http://www.ex-genebank.com/index.php/en/fungi-2); 9) IBT, Culture Collection at Department of Biotechnology and beimedicine, Lyngby, Denmark; 10) IMI/CABI, International Mycological Institute, Kew, England (http://www.cabi.org/services/microbial-services); 11) KAS, fungal collection of Keith A. Seifert, Ottawa, Canada; 12) NRRL, Agricultural Research Service Culture Collection, Peoria, Illinois, USA (https://nrrl.ncaur.usda.gov); 13) UBOCC, Université de Bretagne Occidentale Culture Collection, Brest, France (https://www.univ-brest.fr/plateformes-technologiques/menu/nos-plates-formes/UBOCC); 14) MUT, Mycotheca dell'Università degli

Studi di Torino, Turin, Italy, http://www.mut.unito.it/en/Collezione). Dried holotype and isotype specimens were deposited into the herbarium of the Mycological Department, National Museum, Prague, Czech Republic (PRM) or Canadian National Mycological Herbarium, Ottawa, Canada (DAOM).

Many strains were specifically isolated for the purposes of this study from the indoor environment in the USA, Bermuda, Puerto Rico, Trinidad and Tobago (Ž. Jurjević), house dust from Canada (C.M. Visagie), bakery products and deteriorated paintings in France (M. Coton, F. Déniel), deteriorated paintings from Slovenia (P. Zalar, D.D. Graf) and hypersaline water from Slovenia (P. Zalar, N. Gunde-Cimerman) and Israel (R. Tkavc).

Different isolation techniques were used for species isolation. Samples from indoor environments across the USA were collected using the following techniques: air samples were collected as detailed previously (Peterson & Jurjević 2013) using malt extract agar (MEA) as isolation medium. Dilution plates were used to isolate fungi that were taken by swabs as described previously (Jurjević *et al.* 2015), using MEA supplemented with chloramphenicol and dichloran-glycerol (DG18) agar as isolation medium. Sedimentation plate samples were taken for a one-hour exposure time using Potato Dextrose agar (PDA) as isolation medium. Isolations from Canadian dust were made using a modified dilution-to-extinction method (Collado *et al.* 2007) as described in (Visagie *et al.* 2014, Visagie *et al.* 2017) using DG18, Malt extract Yeast extract 10 % glucose 12 % NaCl (MY10-12) and Malt extract Yeast extract 50 % glucose agar (MY50G) as isolation media.

The samples from paintings in Slovenia were collected as follows. The sampling was performed in 2014 on old (from 300 to 400 years) oil canvas paintings originating from various Slovene churches and at the time of sampling stored in the Restoration Centre for the Protection of Cultural Heritage of Slovenia (ZVKDS). The sampled paintings were partly visibly overgrown with fungi, on the front painted side or on the back of the canvas. Samples were collected by rubbing overgrown areas with sterile cotton swabs soaked in physiological solution [0.9 % (w/v) NaCl]. Inoculum was subsequently spread onto plates containing DG18, MY10-12 or MY50G, all media amended with chloramphenicol (50 mg/l). The plates were incubated at 25 °C for up to 21 d. Pure cultures of the fungi were obtained from the primary isolation plates by further subculturing. Direct isolations were made from fungal growth on deteriorated paintings and bakery products in France using salt malt medium [5 % malt extract, 5 % NaCl, 1.5 % agar, (w/v)] as isolation media. Dilution series were sometimes also used. The paintings were visibly damaged and had been stored in the painting storage area at the Musée des beaux Arts in Brest, France. Plates were incubated at 25 °C for up to 21 d.

Several strains were isolated from different salterns in Slovenia and Israel using filtration of hypersaline water through membrane filters (pore diam 0.45 µm), followed by incubation of the filters on different cultivation media with lowered water activity, as reported by (Gunde-Cimerman *et al.* 2000).

Molecular studies

All isolates included in this study were identified using sequence data, but amplification of four genetic loci (see below) was performed only in 193 isolates selected for phylogenetic analyses (Table 1).

ArchivePure DNA yeast and Gram2+ kit (5 PRIME Inc., Gaithersburg, MD) were used for DNA isolation from 14 d old cultures according to manufacturer instructions as updated by Hubka *et al.* (2015). Target genetic loci, including ITS + LSU rDNA (*ID* region), partial genes encoding calmodulin (*CaM*), β-tubulin (*benA*) and the second largest subunit of RNA polymerase II (*RPB2*), were amplified using primer combinations listed in Table 2. Amplification of *RPB2* with the widely used primers (fRPB2-5F, fRPB2-7CR) designed by Liu *et al.* (1999) was problematic for many sect. *Restricti* isolates. Hence, new primers specific for section *Restricti* (fRPB2ResF100, fRPB2ResR950, Table 2) were designed based on the alignment of available sequences obtained with the Liu *et al.* (1999) primer set. Quality control (hairpin, self-dimer or hetero-dimer formation, melting temperature mismatch) was performed in OligoAnalyzer v. 3.1 (available online http://eu.idtdna.com/calc/analyzer). Standard and touchdown PCR protocols were described previously (Hubka *et al.* 2014, Hubka *et al.* 2016). PCR product purification followed Réblová *et al.* (2016). Automated sequencing was performed at Macrogen Sequencing Service (Amsterdam, The Netherlands) using both terminal primers. Sequences were deposited into GenBank with accession numbers shown in Table 1. All alignments are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3t423.

Phylogenetic analysis

Sequences were inspected in FinchTV (available online http://www.geospiza.com/Products/finchtv.shtml) and assembled in Bioedit v. 7.2.5 (Hall 1999). Alignments were performed using the G-INS-i strategy, as implemented in MAFFT v. 7 (Katoh & Standley 2013). The *benA* alignment contained 431 characters with 227 variable and 217 parsimony informative sites, *CaM* 652 characters with 288 variable and 268 parsimony informative sites, *RPB2* 819 characters with 272 variable and 249 parsimony informative sites, ID 1 123 characters with 231 variable and 175 parsimony informative sites. The concatenated alignment contained 3 025 characters, with 1 018 variable and 909 parsimony informative sites.

Phylogenetic trees based on the concatenated dataset were inferred with both Maximum likelihood (ML) and Bayesian inference (BI) analysis. Partitioning scheme and substitution models for analyses were selected using PartitionFinder v. 1.1.1 (Lanfear *et al.* 2012) with settings allowing introns, exons and codon positions to be independent datasets. Proposed partitioning schemes for each dataset are listed in Table 3. *Hamigera avellanea* NRRL 1938 was used as outgroup.

The ML trees were constructed with IQ-TREE v. 1.4.4 (Nguyen *et al.* 2015) with branch support values obtained from 1 000 bootstrap replicates. Bayesian posterior probabilities (PP) were calculated using MrBayes v. 3.2.6 (Ronquist *et al.* 2012). Optimal partitioning scheme and substitution models were selected using PartitionFinder v. 1.1.1 as described above. The analyses ran for 5 × 10^6 generations, two parallel runs with four chains each were used, every 1 000th tree was retained, and the first 25 % of trees were discarded as burn-in.

For inferring relationships within subg. *Aspergillus*, phylogenies were calculated from a *benA*, *CaM* and *RPB2*

Table 1. Provenance and GenBank accession numbers for *Aspergillus* sect. *Restricti* species used for phylogenetic analysis and comparative phenotypic studies.

Species	Strain no.[1,2]	Source	GenBank accession nos.			
			ITS	*benA*	*CaM*	*RPB2*
A. caesiellus	NRRL 5061^T = CBS 470.65 = DTO 093-H3 = ATCC 11905 = IMI 172278 = CCF 5447 = IBT 34620	Unknown, Japan	EF652044	EF651884	EF652030	EF651981
	NRRL 25810 = CCF 5662	Cloth, Panama	KY087751	KY117814	KY068301	KY117992
	CCF 5450 = EMSL No. 1614	Air, outside, Delaware, USA	KY087598	KY117667	KY068151	KY117844
	CCF 5448 = EMSL No. 1383 = IBT 34621	Air, home, Pennsylvania, USA		KY117665	KY068149	KY117842
	DTO 026-C7	Indoor environment, Germany	KY087684	KY117748	KY068232	KY117925
	DTO 025-I4 = IBT 34538	Indoor environment, Germany	KY087683	KY117747	KY068231	KY117924
	CCF 5451 = EMSL No. 1650 = IBT 34622	Air, pineapple room, warehouse, Delaware, USA	KY087599	KY117668	KY068153	KY117845
	CCF 5449 = EMSL No. 1499	Air, home, Delaware, USA	KY087597	KY117666	KY068150	KY117843
	NRRL 25861	Unknown, Gorakpur, India	KY087766	KY117829	KY068316	KY118007
	NRRL 25815 = DTO 356-D1 = CCF 5663	Hobnail shoes, Florida, USA	KY087753	KY117816	KY068303	KY117994
A. canadensis	CCF 5548^T = KAS 6194 = DTO 356-H9 = IBT 34520 = IBT 34642 = NRRL 66614	House dust, Nova Scotia, Wolfville, Canada	KY087667	KY117731	KY068215	KY117909
	KAS 7705 = DTO 357-A8	House dust, Ottawa, Ontario, Canada	KY087672	KY117736	KY068220	KY117914
	KAS 7707 = DTO 357-B1	House dust, Ottawa, Ontario, Canada	KY087673	KY117737	KY068221	KY117915
	KAS 7708 = DTO 357-B2 = CCF 5550 = IBT 34637	House dust, Ottawa, Ontario, Canada	KY087674	KY117738	KY068222	KY117916
	KAS 7710 = DTO 357-B4 = CCF 5552 = IBT 34636	House dust, Ottawa, Ontario, Canada	KY087675	KY117739	KY068223	KY117917
	KAS 7711 = DTO 357-B5	House dust, Ottawa, Ontario, Canada	KY087676	KY117740	KY068224	KY117918
	KAS 7716 = DTO 357-B8	House dust, Ottawa, Ontario, Canada	KY087677	KY117741	KY068225	KY117919
	KAS 7717 = DTO 357-B9	House dust, Ottawa, Ontario, Canada	KY087678	KY117742	KY068226	KY117920
	KAS 7718 = DTO 357-C1	House dust, Ottawa, Ontario, Canada	KY087679	KY117743	KY068227	KY117921
	KAS 7719 = DTO 357-C2 = CCF 5553 = IBT 34638	House dust, Ottawa, Ontario, Canada	KY087680	KY117744	KY068228	KY117922
	KAS 7704 = DTO 357-A7	House dust, Ottawa, Ontario, Canada	KY087671	KY117735	KY068219	KY117913
	KAS 7721 = DTO 357-C4	House dust, Ottawa, Ontario, Canada	KY087681	KY117745	KY068229	KY117923
A. clavatophorus	NRRL 25874^T = CCF 5454 = IBT 34560 = IBT 34823 = DTO 356-D8	Mouldy paper, Athens, Georgia, USA	KY087772	KY117836	KY068323	KY118014
	NRRL 25873 = CCF 5453 = IBT 34632	Mouldy paper, Athens, Georgia, USA		KY117835	KY068322	KY118013
	DTO 257-G5 = IBT 34561 = CCF 5669	Puerh tea, China	KY087703	KY117764	KY068251	KY117943
A. conicus	NRRL 149^T = CBS 475.65 = IBT 33667 = DTO 096-H6 = ATCC 16908 = IMI 172281 = CCF 5456	Unknown	EF652039	EF651881	EF652033	EF651975
	CCF 5458 = EMSL No. 1490	Air, home, California, USA	KY087601	KY117670	KY068155	KY117846
	CCF 5457 = EMSL No. 1318 = NRRL 62007	Air, home, Idaho, USA	KY087600	KY117669	KY068154	
	NRRL 25881	Unknown, New York, USA	KY087775	KY117839	KY068326	KY118017
	CCF 5459 = EMSL No. 1649	Air, pineapple room, warehouse, Delaware, USA	KY087602	KY117671	KY068156	KY117847
	CCF 5461 = EMSL No. 2549	Air, office, Bayamon, Puerto Rico	KY087604	KY117673	KY068157	KY117849
	CCF 5460 = EMSL No. 2217	Air, living room, West Chester, Pennsylvania, USA	KY087603	KY117672	KY068152	KY117848
	EXF-7663 = IBT 34267 = IBT 33574	Oil painting on canvas, Ljubljana, Slovenia	KY087715	KY117778	KY068265	KY117956
	DTO 077-H5	Indoor air, Witten, The Netherlands	KY087687	KY117751	KY068235	
	IHEM 16531	Wooden statue, Braine-l'Alleud, Belgium	KY087735	KY117798	KY068285	KY117976
	CCF 4042	Kernel of *Bertholletia excelsa*, Czech Republic	KY087659	KY117723	KY068207	KY117903

Table 1. (Continued).

Species	Strain no.[1,2]	Source	GenBank accession nos.			
			ITS	*benA*	*CaM*	*RPB2*
	EXF-7660 = IBT 34263 = IBT 33577	Oil painting on canvas, Ljubljana, Slovenia	KY087713	KY117776	KY068263	KY117954
	EXF-5015 = IBT 34273 = CCF 5650	Microbial mat, Eliat, Israel		KY117774	KY068261	KY117952
	DTO 231-C3	Museum piece, Zwartewaal, The Netherlands	KY087701	KY117763	KY068249	KY117941
	NRRL 25848	Asphalt roof shingle, Chicago, Illinois, USA	KY087764	KY117827	KY068314	KY118005
	IHEM 20709	Candy, Belgium	KY087736	KY117799	KY068286	KY117977
	DTO 110-C5	Air in bathroom, near Copenhagen, Denmark	KY087690	KY117754	KY068238	KY117930
	DTO 110-F5 = IBT 34534 = CCF 5667	Air in living room, near Copenhagen, Denmark	KY087691	KY117755	KY068239	KY117931
	DTO 017-B9	Indoor air, Eindhoven, The Netherlands	KY087682	KY117746	KY068230	
A. destruens	NRRL 145^{T} = CBS 593.91 = DTO 079-A8 = IMI 358691 = CCF 5462 = IBT 34818	Maize seed, Maryland, USA	KY087748	KY117811	KY068298	KY117989
	EXF-7699 = IBT 34262	Oil painting on canvas, Ljubljana, Slovenia	KY087717	KY117780	KY068267	KY117958
	EXF-7651 = IBT 34258 = CCF 5653	Oil painting on canvas, Ljubljana, Slovenia	KY087712	KY117775	KY068262	KY117953
	EXF-7661 = IBT 34271 = IBT 33573	Oil painting on canvas, Ljubljana, Slovenia	KY087714	KY117777	KY068264	KY117955
	DTO 254-B2 = IBT 34522	Air in villa, Utrecht, The Netherlands	KY087702		KY068250	KY117942
	EXF-10411 = IBT 34265	Oil painting on canvas, Ljubljana, Slovenia	KY087724	KY117787	KY068274	KY117965
	EXF-7667 = IBT 34288	Oil painting on canvas, Ljubljana, Slovenia	KY087716	KY117779	KY068266	KY117957
	DTO 220-B2	Air in bakery, Tilburg, The Netherlands	KY087698	KY117760	KY068246	KY117938
	DTO 161-B7 = CCF 5671	Surface of cheese, The Netherlands	KY087697	KY117759	KY068245	KY117937
	NRRL 157 = CCF 5463	Unknown, USA	KY087749	KY117812	KY068299	KY117990
	EXF-10407 = IBT 34285 = CCF 5652	Oil painting on canvas, Ljubljana, Slovenia	KY087723	KY117786	KY068273	KY117964
	EXF-7703 = IBT 34259	Oil painting on canvas, Ljubljana, Slovenia	KY087718	KY117781	KY068268	KY117959
	DTO 147-E2	Indoor air, Hungary	KY087696	KY117758	KY068244	KY117936
	DTO 113-E7 = CCF 5668	Air in bakery, Tilburg, The Netherlands	KY087692	KY117756	KY068240	KY117932
A. domesticus	DTO 079-F2^{T} = CCF 5464 = NRRL 66616 = IBT 34814	Wallpaper, Tiel, The Netherlands	KY087688	KY117752	KY068236	KY117928
	DTO 231-C1 = NRRL 66617 = CCF 5665	Museum piece, Zwartewaal, The Netherlands	KY087700	KY117762	KY068248	KY117940
	DTO 231-B9	Museum piece (mouldy chair backrest), Zwartewaal, The Netherlands	KY087699	KY117761	KY068247	KY117939
	DTO 086-D1 = CCF 5670	Archive material, Gorinchem, The Netherlands	KY087689	KY117753	KY068237	KY117929
	IHEM 6549	Dust from mattress, Brussels, Belgium	KY087734	KY117797	KY068284	KY117975
	EXF-10012 = IBT 34274	Statue made of wood, Textile and sea shells, Ljubljana, Slovenia	KY087719	KY117782	KY068269	KY117960
	UBOCC-A-116022 = CCF 5465	Painting, Brittany, France	KY087605	KY117674	KY068158	KY117850
	CCF 5466 = EMSL No. 1316	Air, home, Idaho, USA	KY087606	KY117675	KY068159	KY117851
A. glabripes	CCF 5474^{T} = EMSL No. 2462 = DTO 356-E8 = NRRL 66618 = IBT 34820	Office folder, Macoya, Trinidad & Tobago	KY087614	KY117683	KY068166	KY117859
	CCF 5473 = EMSL No. 2442 = IBT 34626	Green fabric covered binders, import from China, New Jersey, USA	KY087613	KY117682	KY068165	KY117858
	CCF 5475 = EMSL No. 2463	Office folder, Macoya, Trinidad & Tobago	KY087615	KY117684	KY068167	KY117860

(*continued on next page*)

Table 1. (Continued).

Species	Strain no.[1,2]	Source	GenBank accession nos.			
			ITS	*benA*	*CaM*	*RPB2*
	CCF 5476 = EMSL No. 2464	Office folder, Macoya, Trinidad & Tobago	KY087616	KY117685	KY068168	KY117861
	CCF 5477 = EMSL No. 2465	Office folder, Macoya, Trinidad & Tobago	KY087617	KY117686	KY068169	KY117862
	CCF 5469 = EMSL No. 1483 = DTO 356-E5 = IBT 34519 = IBT 34821 = NRRL 66619	Air, home, California, USA	KY087609	KY117678	KY068161	KY117854
	EMSL No. 1812 = CCF 5470	Front cover of log book, library, Louisiana, USA	KY087610	KY117679	KY068162	KY117855
	EMSL No. 1813 = CCF 5471	Book, library, Louisiana, USA	KY087611	KY117680	KY068163	KY117856
	EMSL No. 1317 = CCF 5468	Air, home, Idaho, USA	KY087608	KY117677	KY068160	KY117853
	EMSL No. 2305 = CCF 5472	Air, kitchen, Summerville, South Carolina, USA	KY087612	KY117681	KY068164	KY117857
	UBOCC-A-116021 = CCF 5467 = IBT 34625	Painting, Brittany, France	KY087607	KY117676		KY117852
A. gracilis	NRRL 4962^T = CBS 539.65 = DTO 351-H7 = CCF 5478 = ATCC 16906 = IMI 211393 = IBT 34817	Gun-firing mechanism, South Pacific	EF652045	EF651883	EF652031	EF651980
	CCF 5479 = EMSL No. 2775 = DTO 356-F4 = IBT 34559	Child carrier, San Diego, California, USA	KY087618	KY117687	KY068170	KY117863
	CCF 5480 = EMSL No. 2920	Child carrier, San Diego, California, USA	KY087619	KY117688	KY068171	KY117864
	CCF 5481 = EMSL No. 2922	Child carrier, San Diego, California, USA	KY087620	KY117689	KY068172	KY117865
	CCF 5482 = EMSL No. 2923 = IBT 34623	Child carrier, San Diego, California, USA	KY087621	KY117690	KY068173	KY117866
A. halophilicus	NRRL 2739^T = ATCC 16401 = CBS 122.62 = IMI 211802 = IBT 34878 = CCF 5687	Dried corn, St. Paul, Minnesota, USA	EF652088	EF651926	EF652034	EF651982
	DTO 271-F4 = CCF 5825 = IBT 34881	Textile, imported into the Netherlands	KY087705	KY117766	KY068253	KY117945
A. hordei	NRRL 25825^T = CCF 5483 = DTO 356-D3 = IBT 34539	Barley, St. Paul, Minnesota, USA	KY087759	KY117822	KY068309	KY118000
	NRRL 25826 = CCF 5484	Barley, St. Paul, Minnesota, USA	KY087760	KY117823	KY068310	KY118001
	NRRL 25830 = CCF 5485 = IBT 34631	Insulating board, St. Paul, Minnesota, USA	KY087761	KY117824	KY068311	KY118002
A. infrequens	NRRL 25868^T = CCF 5486 = DTO 356-D6 = IBT 34524	Wheat, Peoria, Illionois, USA	KY087770	KY117833	KY068320	KY118011
A. magnivesiculatus	NRRL 25866^T = CCF 5488 = IBT 34816	Katsuobushi, Tokyo, Japan	KY087768	KY117831	KY068318	KY118009
	CCF 5491 = EMSL No. 2918	Child carrier, San Diego, California, USA	KY087624	KY117692	KY068176	KY117869
	CCF 5489 = EMSL No. 1315 = DTO 356-E2 = IBT 34516	Air, home, Idaho, USA	KY087622	KY117691	KY068174	KY117867
	CCF 5490 = EMSL No. 2741	Child carrier, San Diego, California, USA	KY087623		KY068175	KY117868
	NRRL 25867 = CCF 5660	Katsuobushi, Tokyo, Japan	KY087769	KY117832	KY068319	KY118010
	NRRL 25821 = CCF 5487	Dried corn, St. Paul, Minnesota, USA	KY087756	KY117819	KY068306	KY117997
	EXF-10353 = IBT 34284	Oil painting on canvas, Ljubljana, Slovenia	KY087720	KY117783	KY068270	KY117961
	KAS 5655 = DTO 356-G8	House dust, Ottawa, Ontario, Canada	KY087661	KY117725	KY068209	KY117905
	KAS 5754 = DTO 356-G9	House dust, Ottawa, Ontario, Canada	KY087662	KY117726	KY068210	
	EXF-10377	Oil painting on canvas, Ljubljana, Slovenia	KY087721	KY117784	KY068271	KY117962
	KAS 5623 = DTO 356-G7	House dust, Stittsville, Ontario, Canada	KY087660	KY117724	KY068208	KY117904
	KAS 6089 = DTO 356-H3	House dust, Wolfville, Nova Scotia, Canada	KY087664	KY117728	KY068212	KY117907
A. pachycaulis	NRRL 25824^T = CCF 5492 = DTO 356-D2 = IBT 34521 = IBT 34812	Unknown, Washington, District of Columbia, USA	KY087758	KY117821	KY068308	KY117999
	CCF 5493 = EMSL No. 2310 = DTO 356-E6 = IBT 34536	Air, home, California, USA	KY087625	KY117693	KY068177	KY117870
A. penicillioides	NRRL 4548^T = CBS 540.65 = ATCC 16910 = IMI 211342 = DTO 207-I7 = CCF 5494 = IBT 34627	Human skin, Recife, Brazil	EF652036	EF651928	EF652024	EF651930
	CCF 5497 = EMSL No. 2430 = IBT 34628	Green fabric covered binders, import from China, New Jersey, USA	KY087626	KY117694	KY068178	KY117871

Table 1. (Continued).

Species	Strain no.[1,2]	Source	GenBank accession nos.			
			ITS	*benA*	*CaM*	*RPB2*
	IHEM 2330	Seeds of cereal, France	KY087730	KY117793	KY068280	KY117971
	IHEM 2476	Indoor air, Brussels, Belgium	KY087732	KY117795	KY068282	KY117973
	DTO 281-A7	Leather, imported into the Netherlands	KY087706	KY117767	KY068254	KY117946
	CCF 3282	Sweet roll with chocolate glaze, Prague, Czech Republic	KY087657	FR775347	HE578103	KY117902
	CBS 140430 = UBOCC-A-115042 = DTO 334-E1	French madeleines, France	KY087596	KY117664	KY068148	KY117841
	CCF 5500 = EMSL No. 2651 = IBT 34630	Baseball gloves, store, O'fallon, Illinois, USA	KY087629	KY117697	KY068181	KY117874
	CCF 5503 = EMSL No. 2909	Child carrier, San Diego, California, USA	KY087632	KY117700	KY068184	KY117877
	NRRL 25816 = CCF 5661	Unknown, Durham, North Carolina, USA	KY087754	KY117817	KY068304	KY117995
	NRRL 25834 = CCF 5659	Peas, St. Paul, Minnesota, USA	KY087762	KY117825	KY068312	KY118003
	NRRL 25835	Wheat, St. Paul, Minnesota, USA	KY087763	KY117826	KY068313	KY118004
	KAS 7745	House dust, Ottawa, Ontario, Canada	KY087746	KY117809	KY068296	KY117987
	KAS 7746	House dust, Ottawa, Ontario, Canada	KY087747	KY117810	KY068297	KY117988
	DTO 267-A9 = CCF 5664	House dust, Micronesia	KY087704	KY117765	KY068252	KY117944
	CCF 2666	Leather shoe, Zlín, Czech republic	KY087655	HE578081	HE578102	KY117900
	CCF 5501 = EMSL No. 2749 = IBT 34629	Child carrier, San Diego, California, USA	KY087630	KY117698	KY068182	KY117875
	CCF 5504 = EMSL No. 3264	Archival cabinet, Bethesda, Maryland, USA	KY087633	KY117701	KY068185	KY117878
	NRRL 25870	Unknown	KY087771	KY117834	KY068321	KY118012
	NRRL 25879	Blood sample, New York, USA	KY087774	KY117838	KY068325	KY118016
	CCF 5499 = EMSL No. 2441	Green fabric covered binders, import from China, New Jersey, USA	KY087628	KY117696	KY068180	KY117873
	NRRL 4550 = CCF 5495	Human skin, Recife, Brazil	EF652037	EF651929	EF652025	EF651931
	NRRL 4553 = CCF 5496	Human skin, Recife, Brazil	KY087750	KY117813	KY068300	KY117991
	CCF 5498 = EMSL No. 2440 = DTO 356-E7 = IBT 34815	Green fabric covered binders, import from China, New Jersey, USA	KY087627	KY117695	KY068179	KY117872
	CCF 5502 = EMSL No. 2900	Child carrier, San Diego, California, USA	KY087631	KY117699	KY068183	KY117876
	NRRL 25820	Dried corn, St. Paul, Minnesota, USA	KY087755	KY117818	KY068305	KY117996
	NRRL 25822	Dried corn, St. Paul, Minnesota, USA	KY087757	KY117820	KY068307	KY117998
A. pseudogracilis	CCF 5505^T = EMSL No. 2765 = DTO 356-F3 = NRRL 66620 = IBT 34813	Child carrier, San Diego, California, USA	KY087634	KY117702	KY068186	KY117879
A. restrictus	NRRL 154^T = CBS 117.33 = CBS 541.65 = DTO 079-B2 = ATCC 16912 = IHEM 3920 = IMI 16267 = IHEM 3920 = CCF 5506 = IBT 34615	Cloth, United Kingdom	EF652042	EF651880	EF652029	EF651978
	CCF 5511 = EMSL No. 1675 = IBT 34616	Packing material, Maryland, USA	KY087639	KY117707	KY068191	KY117884
	NRRL 25862	Culture contaminant, Peoria, Illinois, USA	KY087767	KY117830	KY068317	KY118008
	IHEM 2121	Dust from mattress, Antwerp, Belgium	KY087729	KY117792	KY068279	KY117970
	CCF 5512 = EMSL No. 2206 = IBT 34617	Air, auditorium, school, Sicklerville, New Jersey, USA	KY087640	KY117708	KY068192	KY117885
	CCF 5513 = EMSL No. 2429	Green fabric covered binders, import from China, New Jersey, USA	KY087641	KY117709	KY068193	KY117886
	CCF 5509 = EMSL No. 1611	Mattress cover, North Carolina, USA	KY087637	KY117705	KY068189	KY117882

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Table 1. (Continued).

Species	Strain no.[1,2]	Source	GenBank accession nos.			
			ITS	*benA*	*CaM*	*RPB2*
	DTO 065-C7 = IBT 34541	Corn kernels, Indonesia	KY087685	KY117749	KY068233	KY117926
	CCF 3364 = IBT 34619	Sclerotium of fungus *Corallocytostroma ornicopreoides* imported from Australia, Prague, Czech Republic	KY087658	FR775348	HE578101	HE578109
	CCF 5514 = EMSL No. 2652	Baseball gloves, store, O'fallon, Illinois, USA	KY087642	KY117710	KY068194	KY117887
	NRRL 25882	Cattle feed, USA	KY087776	KY117840	KY068327	KY118018
	CCF 5515 = EMSL No. 2906	Child carrier, San Diego, California, USA	KY087643	KY117711	KY068195	KY117888
	IHEM 818	Indoor air, Estinnes-au-Mont, Belgium	KY087728	KY117791	KY068278	KY117969
	CCF 5510 = EMSL No. 1633	Air, hospital, New Jersey, USA	KY087638	KY117706	KY068190	KY117883
	CCF 5508 = EMSL No. 1416	Air, home, Alabama, USA	KY087636	KY117704	KY068188	KY117881
	CCF 5507 = EMSL No. 1379 = IBT 34618	Air, home, Bermuda	KY087635	KY117703	KY068187	KY117880
	IHEM 2477	Indoor air, Brussels, Belgium	KY087733	KY117796	KY068283	KY117974
	IHEM 2373	Indoor air, Brussels, Belgium	KY087731	KY117794	KY068281	KY117972
A. reticulatus	NRRL 25852^{T} = CCF 5516 = DTO 356-D4 = IBT 34540	Lung biopsy, Charleston, South Carolina, USA	KY087765	KY117828	KY068315	KY118006
	CCF 5523 = EMSL No. 2526	Air, administrative area, Bayamon, Puerto Rico	KY087649	KY117717	KY068201	KY117894
	CCF 5524 = EMSL No. 2548 = IBT 34637	Air, office, Bayamon, Puerto Rico	KY087650	KY117718	KY068202	KY117895
	CCF 5518 = EMSL No. 1272 = NRRL 58903 = IBT 34819	Air, home, Idaho, USA	KY087644	KY117712	KY068196	KY117889
	CCF 5519 = EMSL No. 1313 = NRRL 62004	Air, home, Idaho, USA	KY087645	KY117713	KY068197	KY117890
	CCF 5520 = EMSL No. 1314 = NRRL 62005	Air, home, Idaho, USA	KY087646	KY117714	KY068198	KY117891
	CCF 5521 = EMSL No. 1362 = DTO 356-E4 = IBT 34518	Air, outside, Idaho, USA	KY087647	KY117715	KY068199	KY117892
	CCF 5525 = EMSL No. 885 = NRRL 58572 = IBT 34880	Air, home, Florida, USA	KY087651	KY117719	KY068203	KY117896
	CCF 5522 = EMSL No. 2525	Air, administrative area, Bayamon, Puerto Rico	KY087648	KY117716	KY068200	KY117893
	IHEM 22696	Dust from carpet, Brussels, Belgium	KY087737	KY117800	KY068287	KY117978
	EXF-10429 = CCF 5656	Oil painting on canvas, Ljubljana, Slovenia	KY087725	KY117788	KY068275	KY117966
	CCF 3112 = IBT 34634 = NRRL 62490	Leather shoe, Zlín, Czech Republic	KY087656	FR775323	FR751451	KY117901
	NRRL 25878 = CCF 5517	Lung biopsy, Chamblee, Georgia, USA	KY087773	KY117837	KY068324	KY118015
A. salinicola	EXF-10401^{T} = IBT 34266 = CCF 5526 = NRRL 66621	Oil painting on canvas, Ljubljana, Slovenia	KY087722	KY117785	KY068272	KY117963
	KAS 6054	House dust, Wolfville, Nova Scotia, Canada	KY087738	KY117801	KY068288	KY117979
	UBOCC-A-116019 = CCF 5528 = IBT 34635	Painting, Brittany, France	KY087652	KY117720	KY068204	KY117897
	EXF-226 = CCF 5527 = IBT 34277 = NRRL 66622	Hypersaline water from salterns, Sečovlje salterns, Slovenia	KY087711	KY117773	KY068260	
A. tardicrescens	DTO 316-B5^{T} = CCF 5529 = IBT 34558 = NRRL 66623	Museum piece (measuring table), Alphen aan den Rijn, The Netherlands	KY087710	KY117772	KY068259	KY117951
	DTO 316-A7	Museum piece (dentist chair), Alphen aan den Rijn, The Netherlands	KY087707	KY117768	KY068255	KY117947
	DTO 316-A8 = IBT 34562	Museum piece (rubber tyre of brancard), Alphen aan den Rijn, The Netherlands	KY087708	KY117769	KY068256	KY117948
	DTO 316-A9	Museum piece (x-ray table), Alphen aan den Rijn, The Netherlands		KY117770	KY068257	KY117949
	DTO 316-B4	Museum piece (vitrine), Alphen aan den Rijn, The Netherlands	KY087709	KY117771	KY068258	KY117950

Table 1. (Continued).

Species	Strain no.[1,2]	Source	GenBank accession nos.			
			ITS	*benA*	*CaM*	*RPB2*
	EXF-10456 = IBT 34286	Air in depot of Conservation Centre of the Institute for the Protection of Cultrural Heritage of Slovenia, Ljubljana, Slovenia	KY087727	KY117790	KY068277	KY117968
	KAS 6252 = DTO 356-I5	House dust, Wolfville, Nova Scotia, Canada	KY087669	KY117733	KY068217	KY117911
	EXF-10454 = IBT 34281 = CCF 5530 = NRRL 66624	Oil painting on canvas, Ljubljana, Slovenia	KY087726	KY117789	KY068276	KY117967
	DTO 073-H6	Moist wall of archive, Tilburg, The Netherlands	KY087686	KY117750	KY068234	KY117927
A. villosus	NRRL 25813^T = CCF 5531 = DTO 356-C9 = IBT 34822	Unknown, Kirkhill, Scotland, United Kingdom	KY087752	KY117815	KY068302	KY117993
	UBOCC-A-116020 = CCF 5532 = IBT 34624	Painting, Brittany, France	KY087653	KY117721	KY068205	KY117898
A. vitricola	NRRL 5125^T = DTO 356-F7 = ATCC 16905 = ATCC 36505 = IMI 108298 = CCF 5533 = IBT 34530	Binocular lens, Japan	EF652046	EF651927	EF652035	EF651973
	KAS 6086	House dust, Little Lepreau, New Brunswick, Canada	KY087739	KY117802	KY068289	KY117980
	KAS 6281	House dust, Victoria, British Columbia, Canada	KY087744	KY117807	KY068294	KY117985
	KAS 6087 = DTO 356-H2 = IBT 34532	House dust, Victoria, British Columbia, Canada	KY087663	KY117727	KY068211	KY117906
	KAS 6238	House dust, Victoria, British Columbia, Canada	KY087743	KY117806	KY068293	KY117984
	KAS 6199	House dust, Victoria, British Columbia, Canada	KY087742	KY117805	KY068292	KY117983
	KAS 6288	House dust, Wolfville, Nova Scotia, Canada	KY087745	KY117808	KY068295	KY117986
	KAS 6237 = DTO 356-I2	House dust, Victoria, British Columbia, Canada	KY087668	KY117732	KY068216	KY117910
	KAS 6133 = DAOMC 251500	House dust, Little Lepreau, New Brunswick, Canada	KY087741	KY117804	KY068291	KY117982
	KAS 6093	House dust, Victoria, British Columbia, Canada	KY087740	KY117803	KY068290	KY117981
	DTO 122-I4	Archive material, Gorinchem, The Netherlands	KY087693	KY117757	KY068241	KY117933
	DTO 123-B2	Archive material, Gorinchem, The Netherlands	KY087695		KY068243	KY117935
	DTO 122-I5	Archive material, Gorinchem, The Netherlands	KY087694		KY068242	KY117934
	KAS 6278 = DTO 356-I8	House dust, Wolfville, Nova Scotia, Canada	KY087670	KY117734	KY068218	KY117912
	KAS 6150 = DTO 356-H5 = IBT 34531	House dust, Wolfville, Nova Scotia, Canada	KY087665	KY117729	KY068213	
	KAS 6178 = DTO 356-H8	House dust, Little Lepreau, New Brunswick, Canada	KY087666	KY117730	KY068214	KY117908
	CCF 5534 = EMSL No. 2785	Child carrier, San Diego, California, USA	KY087654	KY117722	KY068206	KY117899

[1] Acronyms of culture collections: ATCC, American Type culture collection, Manassas, Virginia, USA; CBS, culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCF, Culture Collection of Fungi, Charles University, Czech Republic; DAOMC, Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada; DTO, working collection of the department of Applied and Industrial Mycology housed at CBS; EMSL, EMSL Analytical Inc., New Jersey, USA; EXF, Culture Collection of Extremophilic Fungi, University of Ljubljana, Slovenia; IBT, Culture Collection at Center for Microbial Biotechnology, Lyngby, Denmark; BCCM/IHEM, Biomedical Fungi and Yeasts Collection, Scientific Institute of Public Health, Brussels, Belgium; IMI/CABI, International Mycological Institute, Kew, England; KAS, fungal collection of Keith A. Seifert, Ottawa, Canada; NRRL, Agricultural Research Service Culture Collection, Peoria, Illinois, USA; UBOCC, Université de Bretagne Occidentale Culture Collection, Brest, France.

[2] Ex-type strains are designated with superscript T.

concatenated dataset containing 102 individuals and 1 902 characters, of which 929 characters were variable and 864 were parsimony informative. ML and BI analyses were inferred as described above. Suitable partitioning schemes selected using PartitionFinder v. 1.1.1 are listed in Table 3.

Species delimitation and species tree inference

Nucleotide substitution models for each locus were determined using jModeltest v. 2.1.7 (Posada 2008) based on the Bayesian information criterion (BIC) and are listed in Table 4.

Table 2. Primers used for amplification and sequencing.

Locus	Primer	Orientation	Sequence (from 5′ to 3′)	References
benA	Bt2a	Forward	GGTAACCAAATCGGTGCTGCTTTC	Glass & Donaldson (1995)
	T10	Forward	ACGATAGGTTCACCTCCAGAC	O'Donnell & Cigelnik (1997)
	Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	Glass & Donaldson (1995)
CaM	CF1L	Forward	GCCGACTCTTTGACYGARGAR	Peterson (2008)
	CF1M	Forward	AGGCCGAYTCTYTGACYGA	Peterson (2008)
	cmd5	Forward	CCGAGTACAAGGAGGCCTTC	Hong *et al.* (2006)
	CF4	Reverse	TTTYTGCATCATRAGYTGGAC	Peterson (2008)
	cmd6	Reverse	TTTYTGCATCATRAGYTGGAC	Hong *et al.* (2006)
RPB2	fRPB2-5F	Forward	GAYGAYMGWGATCAYTTYGG	Liu *et al.* (1999)
	fRPB2ResF100	Forward	TGAARTAYGCICTTGCYAC	Newly designed
	fRPB2-7CR	Reverse	CCCATRGCTTGYTTRCCCAT	Liu *et al.* (1999)
	fRPB2ResR950	Reverse	CARTGYGTCCADGTRTGKGC	Newly designed
ITS	ITS1	Forward	TCCGTAGGTGAACCTGCGG	White *et al.* (1990)
	NL4	Reverse	GGTCCGTGTTTCAAGACGG	O'Donnell (1993)
	ITS4	Reverse	TCCTCCGCTTATTGATATGC	White *et al.* (1990)

To assign individuals into species, several species delimitation methods were employed. In all cases the alignment was split into two parts. The first part contained the *A. restrictus*, *A. conicus* and *A. vitricola* clades, the second part the *A. penicillioides* clade. The Bayesian version of the general mixed yule-coalescent model (bgmyc) was performed in R v. 3.3.1 (R Core Team 2015) with the bGMYC package (Reid & Carstens 2012). The general mixed yule-coalescent method (GMYC) was performed in R v. 3.3.1 using the splits package (Fujisawa & Barraclough 2013). Single-locus ultrametric trees created in BEAST v. 2.4.2 (Bouckaert *et al.* 2014) were used as an input for both methods. Chain length for each tree was 1×10^7 generations with 25 % burn-in. The highest credibility tree was used for the GMYC method and 100 trees sampled throughout the analysis were used for the bGMYC method. These trees were obtained by equal sampling of all the trees from the analysis after discarding the first 60 % of trees. The analysis according Poisson tree processes (PTP) model was performed on species delimitation server (Zhang *et al.* 2013). The method does not require an ultrametric tree, so the single-locus input trees were calculated using ML analysis in IQ-TREE web server (Trifinopoulos *et al.* 2016). Species delimitation using the Automatic barcode gap discovery (ABGD) was performed on ABGD web (Puillandre *et al.* 2012). Finally multilocus species delimitation (STACEY) was performed with the BEAST v. 2.4.2 add-on STACEY v. 1.2.2 (Jones 2017). The chain length was set to 5×10^8 generations, priors were set as follows: the species tree prior was set to the Yule model, growth rate prior was set to lognormal distribution (M = 5, S = 2), clock rate priors for all loci were set to lognormal distribution (M = 0, S = 1), PopPriorScale prior was set to lognormal distribution (M = -7, S = 2) and relativeDeathRate prior was set to beta distribution (α = 1, β = 1 000). The output was processed with SpeciesDelimitationAnalyzer (Jones 2017).

Species trees were inferred using *BEAST (Heled & Drummond 2010) implemented in BEAST v. 2.4.2. (Bouckaert *et al.* 2014). Individuals were assigned into species based on the consensual results from the above-mentioned species delimitation methods. The MCMC analysis was run for 1×10^8 of generations, 25 % of trees was discarded as burn-in. Strict

Table 3. Partition-merging results and best substitution model for each partition according to Bayesian information criterion as proposed by PartitionFinder v1.1.1.

Dataset	Phylogenetic method[1]	Partitioning scheme (substitution model)
Sect. *Restricti* (ITS + *benA* + *CaM* + *RPB2*)	ML	*benA* + *CaM* introns (HKY+I+G); 1st codon positions of *benA* + *CaM* + *RPB2* + 2nd codon positions of *RPB2* (TrNef+I+G); ITS + LSU (TrNef+I+G); 2nd codon positions of *benA* + *CaM* + 3rd codon positions of *benA* + *CaM* (TIM+G); 3rd codon positions of *RPB2* (HKY+G)
	BI	*benA* + *CaM* introns (HKY+I+G); 1st codon positions of *benA* + *CaM* + 2nd codon positions of *RPB2* (K80+I+G); 2nd codon positions of *benA* + *CaM* + 3rd codon positions of *benA* (SYM+G); 1st codon positions of *RPB2* + 3rd codon positions of *CaM* + ITS + LSU (GTR+I+G); 3rd codon positions of *RPB2* (HKY+G)
Subg. *Aspergillus* (*benA* + *CaM* + *RPB2*)	ML	*benA* + *CaM* introns (HKY+I+G); 1st codon positions of *benA* (JC+I); 2nd codon positions of *benA* + *CaM* + *RPB2* (F81+I); 3rd codon positions of *benA* + *CaM* (GTR+G); 1st codon positions of *CaM* + *RPB2* (TrN+I+G); 3rd codon positions of *RPB2* (TrNef+G)
	BI	*benA* + *CaM* introns (HKY+I+G); 1st codon positions of *benA* (JC+I); 2nd codon positions of *benA* + *CaM* + *RPB2* (F81+I); 3rd codon positions of *benA* + *CaM* (GTR+G); 1st codon positions of *CaM* + *RPB2* (GTR+I+G); 3rd codon positions of *RPB2* (HKY+G)

[1] ML, Maximum likelihood; BI, Bayesian inferrence.

Table 4. Nucleotide substitution models selected by jModeltest 2.1.7 for each locus according to Bayesian information criterion.

Clade	Locus	Selected substitution model
A. restrictus, *A. conicus*, *A. vitricola* clades	*benA*	SYM + G
	CaM	TrNef + G
	RPB2	K80 + G
	ITS + LSU	TrN + I
A. penicillioides clade	*benA*	TrNef + G
	CaM	TrNef + I
	RPB2	TrNef + G
	ITS + LSU	SYM + I + G
Whole dataset	*benA*	TrNef + G
	CaM	TrNef + G
	RPB2	TrNef + I + G
	ITS + LSU	TrNef + I + G

molecular clock was chosen for all loci and population function was set as constant. Convergence was assessed by examining the likelihood plots in Tracer v. 1.6 (Rambaut *et al.* 2014).

Species delimitation hypotheses were tested by a coalescent-based approach implemented in BP&P v. 3.1 (Bayesian phylogenetics and phylogeography) (Yang & Rannala 2010). Species delimitation using rjMCMC (reversible jump MCMC algorithm allows to inspect different models with given species tree) was performed with similar isolates allocation to species as during the species tree inference and the tree topology created according to the results from *BEAST. We analysed three combinations on the prior distributions of the parameters ϑ (ancestral population size) and τ_0 (root age) as proposed by Leaché & Fujita (2010), i.e. large ancestral population sizes and deep divergence: ϑ ~ G (1, 10) and τ_0 ~ G (1, 10); small ancestral population sizes and shallow divergences among species: ϑ ~ G (2, 2 000) and τ_0 ~ G (2, 2 000); large ancestral populations sizes and shallow divergences among species: ϑ ~ G (1, 10) and τ_0 ~ G (2, 2 000).

Species boundaries were further validated by calculating the genealogical sorting index (GSI) (Cummings *et al.* 2008) which quantifies the degree of exclusive ancestry of hypothetical species. In order to perform the analysis, 100 trees inferred from each locus were created using RAxML (Stamatakis *et al.* 2008) with the bootstrap option. Calculation of *gsi* statistics was performed at http://www.molecularevolution.org, with 1 × 10^4 permutations for evaluation of statistical significance.

Comparison with 454 sequence data

Reference sequences generated in this study were compared to 454-pyrosequences obtained from house dust collected during a world-wide survey (Amend *et al.* 2010). Information with regards to dust collection and metagenomic analyses methods, readers are refered to Amend *et al.* (2010). For our comparisons, 454-sequences belonging to sect. *Restricti* were harvested by firstly doing a BLAST search of ITS barcodes from sect. *Restricti* against the main 454 database and retaining all sequences with at least 90 % similarity. This dataset was aligned in MAFFT v. 7 using the G-INS-i algorithm and subsequent neighbour-joining tree calculated in MEGA v. 7 (Kumar *et al.* 2016). This tree was used to remove all sequences that do not belong in sect. *Restricti*. The dataset was subsequently re-aligned and neighbour-joining tree calculated using *Hamigera avellanea* as outgroup.

Morphology

Macromorphological characters of colonies were observed on Harrold's agar (M40Y) (Harrold 1950), Czapek yeast extract agar (CYA) (Pitt 1979), CYA supplemented with 20 % sucrose (CY20S) (Klich 2002), dichloran 18 % glycerol agar (DG18) (Hocking & Pitt 1980), malt extract agar (MEA; Oxoid) (Samson *et al.* 2010), Harrold's agar supplemented with 60 % Sucrose (M60Y) (Raper & Fennell 1965) and MEA supplemented with 10 % NaCl (MEA + 10 % NaCl). The isolates were inoculated in three points on 90 mm Petri dishes and incubated for 14 d at 25 °C in darkness. In addition, CY20S and M60Y plates were incubated at 30 °C and 37 °C. Colony diameters were measured after 14 d of incubation. The colony shape and texture, degree of sporulation, obverse and reverse colony colours, the production of soluble pigments and exudates were determined. The isolates of *A. halophilicus* were cultivated on Czapek–Dox agar (Thom & Church 1926) supplemented with 70 % sucrose (CZA70S) for 30 d. Colour names and codes used in descriptions refer to Kornerup & Wanscher (1967).

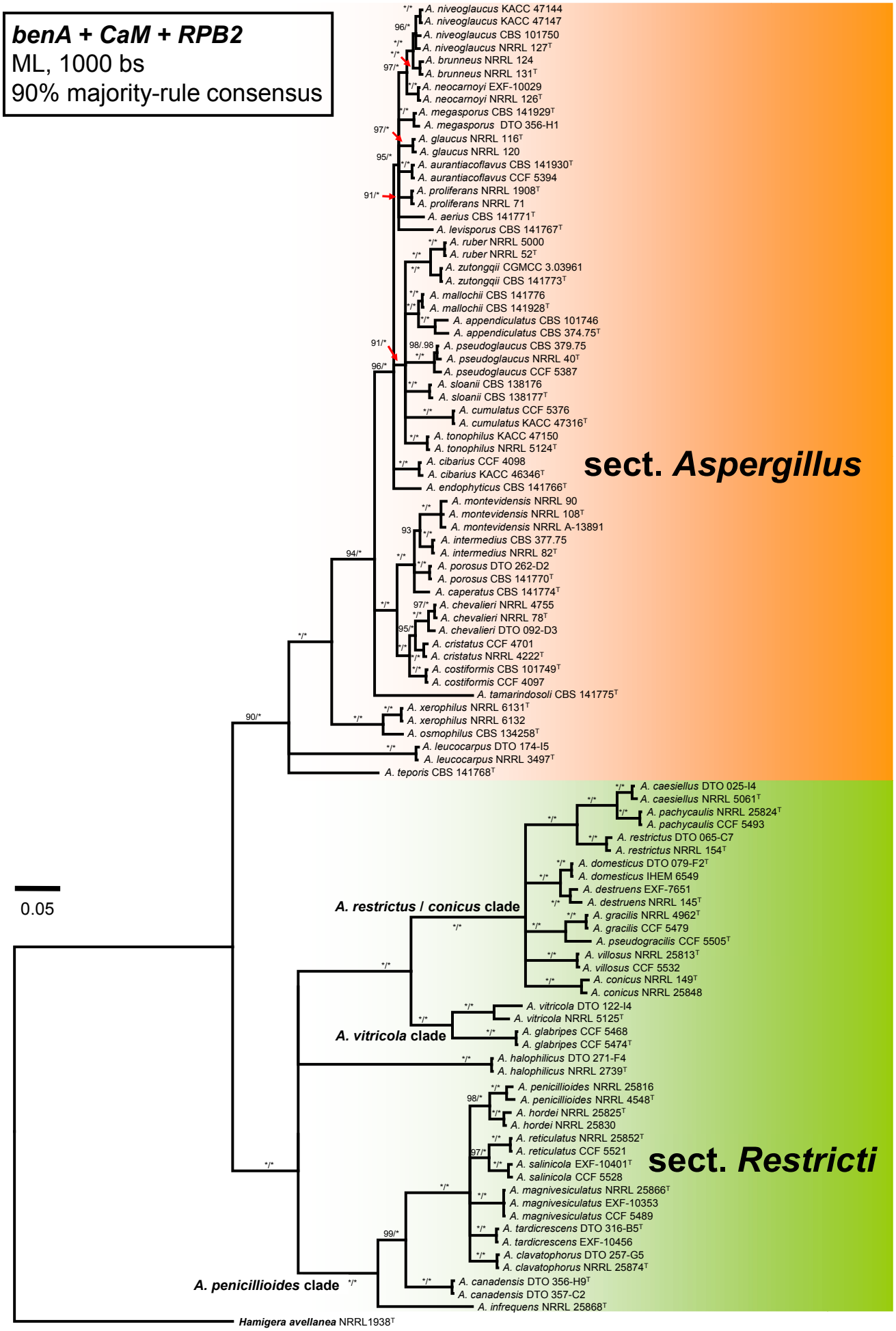
Light microscope preparations were made from 14 d old colonies grown on M40Y. Lactic acid (60 %) was used as mounting fluid and ethanol (96 %) used to remove excess conidia and prevent air bubble formation. Microphotography was done using an Olympus BX-51 microscope with an Olympus DP72 camera and Zeiss AX10 Imager A2 light microscope equipped with a Nikon DS-Ri2 camera. Macromorphology of the colonies was observed and captured on a Zeiss Stereo Discovery V20 dissecting microscope equipped with a Nikon DS-Ri2 camera. Pictures were processed and photographic plates prepared in Adobe Photoshop CS6. Micromorphological characters (length and width of conidia, width of stipes and vesicles and length of phialides) were measured from at least five isolates of each species (when available). Slides were prepared from both the colony centre and margins. Each character was recorded at least forty times for each character and isolate. Linear discriminant analysis was performed with measured data in R 3.3.1 (R Core Team 2015) with packages MASS (Venables & Ripley 2002) and ggplot2 (Wickham 2009). The isolates were assigned to groups based on the results of molecular phylogenetic analyses (see above).

Scanning electron microscopy (SEM) was performed using a JEOL-6380 LV microscope (JEOL Ltd. Tokyo, Japan) as described previously (Hubka *et al.* 2013).

In brief, plugs from colonies (5 × 5 mm) grown 14 d on M40Y containing conidiophores, and ascomata in the case of *A. halophilicus* (longer incubation on CZA70S was necessary) were fixed in osmium tetroxide vapours for 2 wk at 5–10 °C and gold-coated in a Bal-Tec SCD 050 sputter coater. The specimens were observed with spot size 40–42 µm and accelerating voltage 25 kV. Terminology of the surface ornamentation of the conidia was adopted from Kozakiewicz (1989).

Physiology

At least five isolates from each species, when available, were selected for determining species growth rates in an osmotic


benA + CaM + RPB2
ML, 1000 bs
90% majority-rule consensus
0.05
A. niveoglaucus KACC 47144
A. niveoglaucus KACC 47147
A. niveoglaucus CBS 101750
A. niveoglaucus NRRL 127T
A. brunneus NRRL 124
A. brunneus NRRL 131T
A. neocarnoyi EXF-10029
A. neocarnoyi NRRL 126T
A. megasporus CBS 141929T
A. megasporus DTO 356-H1
A. glaucus NRRL 116T
A. glaucus NRRL 120
A. aurantiacoflavus CBS 141930T
A. aurantiacoflavus CCF 5394
A. proliferans NRRL 1908T
A. proliferans NRRL 71
A. aerius CBS 141771T
A. levisporus CBS 141767T
A. ruber NRRL 5000
A. ruber NRRL 52T
A. zutongqii CGMCC 3.03961
A. zutongqii CBS 141773T
A. mallochii CBS 141776
A. mallochii CBS 141928T
A. appendiculatus CBS 101746
A. appendiculatus CBS 374.75T
A. pseudoglaucus CBS 379.75
A. pseudoglaucus NRRL 40T
A. pseudoglaucus CCF 5387
A. sloanii CBS 138176
A. sloanii CBS 138177T
A. cumulatus CCF 5376
A. cumulatus KACC 47316T
A. tonophilus KACC 47150
A. tonophilus NRRL 5124T
A. cibarius CCF 4098
A. cibarius KACC 46346T
A. endophyticus CBS 141766T
sect. Aspergillus
A. montevidensis NRRL 90
A. montevidensis NRRL 108T
A. montevidensis NRRL A-13891
A. intermedius CBS 377.75
A. intermedius NRRL 82T
A. porosus DTO 262-D2
A. porosus CBS 141770T
A. caperatus CBS 141774T
A. chevalieri NRRL 4755
A. chevalieri NRRL 78T
A. chevalieri DTO 092-D3
A. cristatus CCF 4701
A. cristatus NRRL 4222T
A. costiformis CBS 101749T
A. costiformis CCF 4097
A. tamarindosoli CBS 141775T
A. xerophilus NRRL 6131T
A. xerophilus NRRL 6132
A. osmophilus CBS 134258T
A. leucocarpus DTO 174-I5
A. leucocarpus NRRL 3497T
A. teporis CBS 141768T
A. caesiellus DTO 025-I4
A. caesiellus NRRL 5061T
A. pachycaulis NRRL 25824T
A. pachycaulis CCF 5493
A. restrictus DTO 065-C7
A. restrictus NRRL 154T
A. domesticus DTO 079-F2T
A. domesticus IHEM 6549
A. destruens EXF-7651
A. destruens NRRL 145T
A. gracilis NRRL 4962T
A. gracilis CCF 5479
A. pseudogracilis CCF 5505T
A. villosus NRRL 25813T
A. villosus CCF 5532
A. conicus NRRL 149T
A. conicus NRRL 25848
A. restrictus / conicus clade
A. vitricola DTO 122-I4
A. vitricola NRRL 5125T
A. glabripes CCF 5468
A. glabripes CCF 5474T
A. vitricola clade
A. halophilicus DTO 271-F4
A. halophilicus NRRL 2739T
A. penicillioides NRRL 25816
A. penicillioides NRRL 4548T
A. hordei NRRL 25825T
A. hordei NRRL 25830
A. reticulatus NRRL 25852T
A. reticulatus CCF 5521
A. salinicola EXF-10401T
A. salinicola CCF 5528
A. magnivesiculatus NRRL 25866T
A. magnivesiculatus EXF-10353
A. magnivesiculatus CCF 5489
A. tardicrescens DTO 316-B5T
A. tardicrescens EXF-10456
A. clavatophorus DTO 257-G5
A. clavatophorus NRRL 25874T
sect. Restricti
A. canadensis DTO 356-H9T
A. canadensis DTO 357-C2
A. infrequens NRRL 25868T
A. penicillioides clade
Hamigera avellanea NRRL1938T

gradient. Each strain was cultivated at 25 °C on MEA with six different concentrations of NaCl ranging from 0 to 25 %. After 14 d, colony sizes were recorded and growth curves for each species calculated using local regression (LOESS) in R v. 3.3.1. (R Core Team 2015) using the ggplot2 package (Wickham 2009).

Exometabolite analysis

The isolates of *Aspergillus* sect. *Restricti* were incubated on DG18, CY20S and yeast extract sucrose agar (YES) agar, for 2 wk at 25 °C. Two agar plugs from each medium (6 plugs in total) were combined in one vial and extrolites extracted with ethylacetate / isopropanol (3:1) with added 1 % formic acid, and ultrasonication (50 min). In the case of *A. halophilicus*, six CZA70S plugs containing ascomata (ca 1–2 mo old colonies) were extracted. After ultrasonication, the plugs were removed and the organic solvent evaporated. The remaining extract was re-dissolved in methanol, centrifuged at 13 300 rpm and transferred to a small vial with a V-formed insert. HPLC analysis was done according to Frisvad & Thrane (1987) as modified by Nielsen *et al.* (2011).

RESULTS

Phylogeny of subgenus *Aspergillus*

ML and BI analysis of 102 concatenated sequences of *benA*, *CaM* and *RPB2* contained 31 species from sect. *Aspergillus* as recognised by Chen *et al.* (2017) and 21 species from sect. *Restricti* recognised here (see below). Tree topologies between ML and BI did not differ and the ML tree was used with both bootstrap and pp values included (Figs 1 and 2). Both analyses supported the monophyly of both sections. Each section contains several highly supported clades, but the exact position of species within the clades is often unresolved. Despite producing a eurotium-like sexual state common in sect. *Aspergillus*, *A. halophilicus* is resolved with high statistical support in sect. *Restricti*, but its exact position remains unclear. It is apparent from the radial representation of the tree (Fig. 2), that there are large genetic distances between the different clades of sect. *Restricti*, but these gaps may represent only hidden variability that has not been discovered during our study due to insufficient sampling or the use of inappropriate isolation media. The retaining of the current classification scheme of subg. *Aspergilllus* with two sections seems currently the best solution until more data on species diversity in sect. *Restricti* are collected. Additionally, sects. *Restricti* and *Aspergillus* are well supported by phenotypic data (see discussion).

Species delimitation and validation in sect. *Restricti*

For species delimitation, the alignment was divided into two parts as discussed earlier. Eleven species were delimited within the first part that consisted of the *A. restrictus*, *A. conicus* and *A. vitricola* clades using STACEY and similarly, nine species

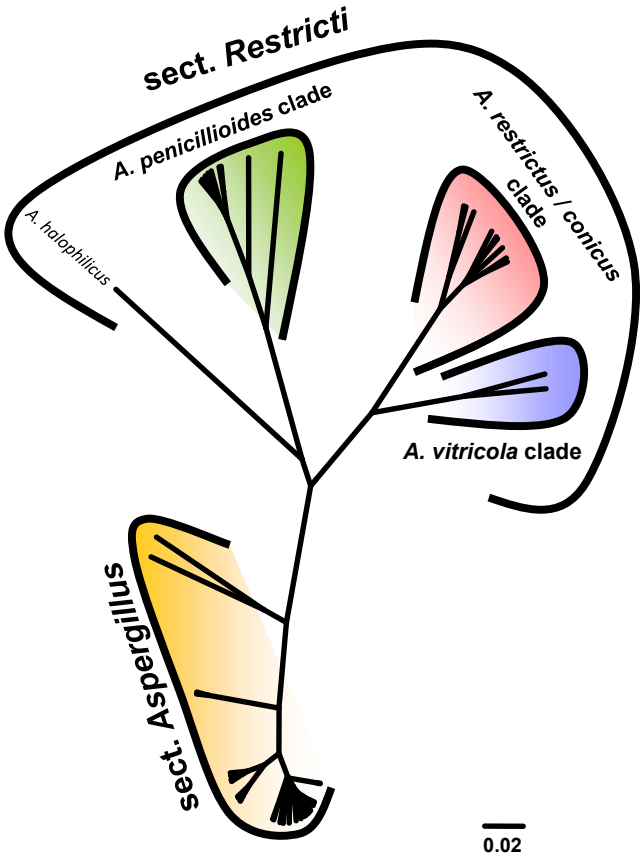


Fig. 2. Maximum likelihood phylogenetic tree of the subgenus *Aspergillus* inferred from partitioned analysis of concatenated dataset (*benA*, *CaM* and *RPB2*) with Maximum likelihood method and presented in radial format.

were delimited within the second part of the data set (*A. penicillioides* clade). *Aspergillus halophilicus* was exluded from species delimitation analyses because it clearly represents a distantly related clade within the section. Results are summarised in Figs 3 and 4. Tree topologies in the Figs 3 and 4 were inferred in STACEY and used solely for the comprehensive presentation of the results from different methods; the evolutionary relationships in the section inferred by *BEAST are presented as the most robust (Fig. 5). In the first step, we compared results from four single-locus species delimitation methods with those derived from STACEY, that is currently one of the most advanced species delimitation methods because it processes multiple loci simultaneously during a single analysis (Jones 2017). Although the results vary across the methods and loci, the consensual results from single-locus species delimitation methods are generally in agreement with the results of STACEY. Single-locus method bGMYC was the most computationally intensive method among those used in this study and its results were most similar to STACEY. The method with greatest variability across the four loci was GMYC with 14 to 39 delimited species for the first part of the analysed data set (*A. restrictus*, *A. conicus* and *A. vitricola* clades) and eight to 38 species for *A. penicillioides* clade. A significant over delimitation was observed when analysing the ID region of the first part of the data set and also *CaM* and *RPB2* loci using GMYC method (Fig. 3). A

Fig. 1. A 90 % majority consensus tree of the subgenus *Aspergillus* inferred with Maximum likelihood analysis based on *benA*, *CaM* and *RPB2* loci (partitioning scheme and substitution models are listed in Table 3). The data set contained 102 strains and 1902 characters, of which 929 characters were variable and 864 were parsimony informative. Support values represent maximum likelihood bootstrap/ Bayesian posterior probability values, 100 % bootstrap values and 1.00 posterior probability are designated by asterisk *. The ex-type isolates are designated by a superscript T. *Hamigera avellanea* (NRRL 1938) was used as outgroup.

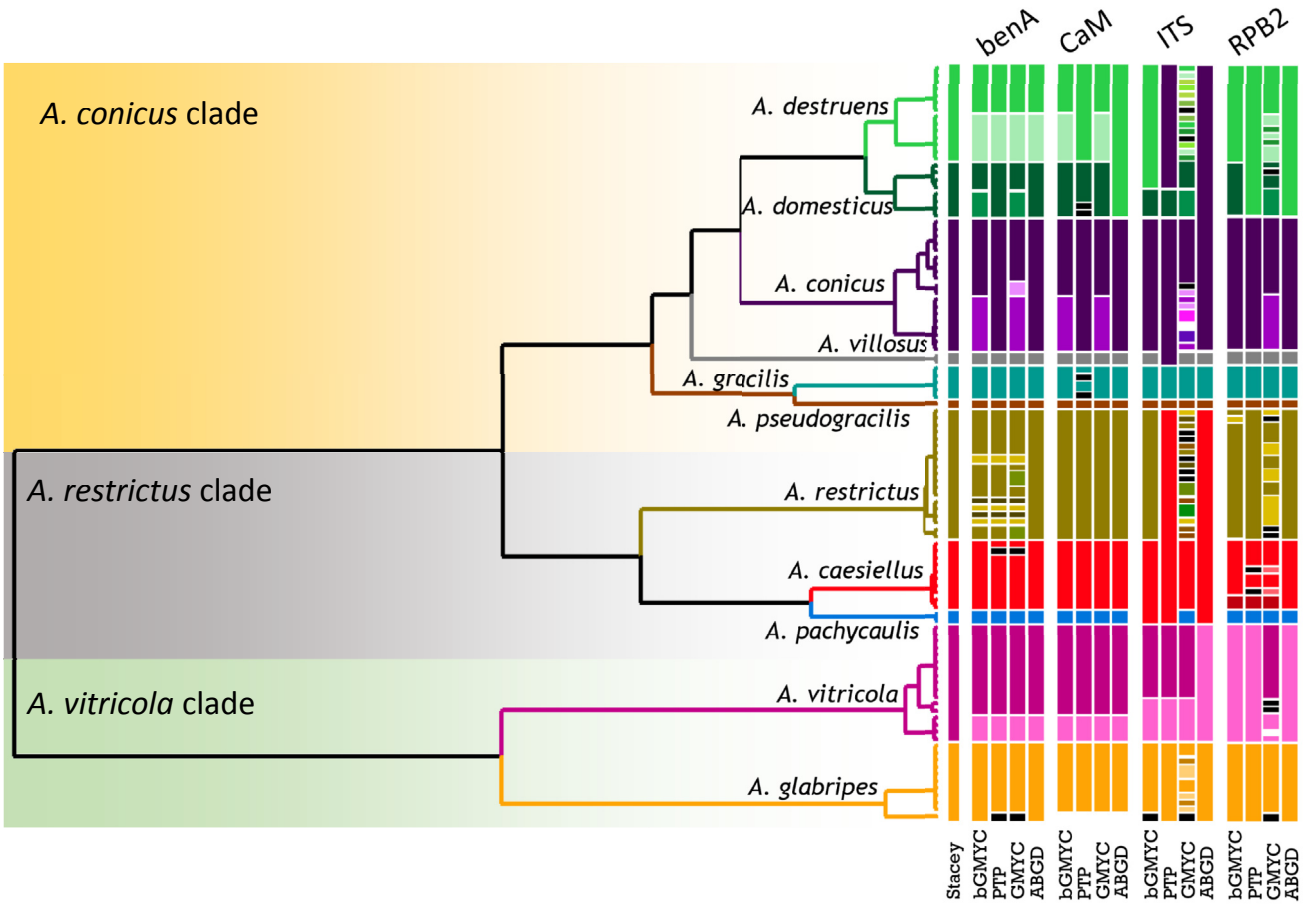


Fig. 3. Schematic representation of results of species delimitation methods in *A. restrictus*, *A. conicus* and *A. vitricola* clades (108 isolates). The results of multilocus method (STACEY) are compared to results of single-locus methods (bGMYC, PTP, GMYC, ABGD). Results from different methods are depicted with coloured bars highlighting congruence across methods. Displayed tree comes from STACEY analysis and is used solely for the comprehensive presentation of the results from different methods.

similar problem was observed in the case of *CaM* when analysing the second part of the data set and also in *RPB2* locus when using GMYC method (Fig. 4). Although ABGD is a quite simplistic method compared to other used methods it yielded similar results to STACEY and bGMYC but over delimitation was observed when analysing the *CaM* locus in the *A. penicillioides* clade (Figs 3–4). The number of species delimited by PTP was slightly higher compared to bGMYC, but lower than in the case of GMYC. Single-locus methods often delimited additional species within *A. conicus* and *A. vitricola*, but the results were not consistent and in some cases even contradictory, suggesting recombination within the clade (Fig. 3). These tentative species had no or very limited phenotypic support, which is the reason for adopting a broader species concept. In contrast, the majority of single-locus methods did not support delimitation of *A. clavatophorus* and *A. pachycaulis* based on the ID region; the methods also did not support recognition of *A. destruens* and *A. domesticus* when analysing ID-region and *RPB2* in contrast to *benA* and *CaM*. All these species were supported by STACEY and phenotype analysis, resulting in us proposing them as new species.

Based on consensus results of species delimitation methods and after reflection of phenotypic data in ambiguous species, we recognise 21 species within the sect. *Restricti*. This number comprises seven previously recognised and 14 new species proposed here (see section Taxonomy). All four loci have sufficient variability for reliable species identification and can be used as DNA barcodes. ID region has the lowest discriminative power but it is still sufficient for differentiation of all species. The locus with the highest ratio of variable positions to the sequence length was *benA*.

The species validation analysis results are listed in Table 5. All species were supported by the posterior probability 1.00 based on the analysis in BP&P v. 3.1 (Yang & Rannala 2010) under all three scenarios simulated by different prior distributions of parameters ϑ (ancestral population size) and τ_0 (root age). The gsi calculations and significance testing performed using the genealogical sorting index software also confirmed that all delimited species can be considered separate evolutionary lineages. The ensemble statistic gsi_T (weighted sum of gsi across genealogies) for each dataset (100 bootstrap trees for each locus and concensus trees) are listed in Table 5. Relatively low gsi value was obtained from the analysis of β-tubulin dataset in *A. hordei* and ID dataset in *A. domesticus*, however even in these cases the p-value of the permutation test is nearly 0. The *gsi* statistic was not calculated for *A. infrequens* and *A. pseudogracilis* because they were both represented by only one isolate.

Species tree

The species tree topology was inferred with *BEAST (Heled & Drummond 2010) and is depicted on Figs 5 and 6. It was used as a guide tree for species validation in BP&P but it also represents the most probable evolutionary relationships between the species. Four clusters of species designated as the

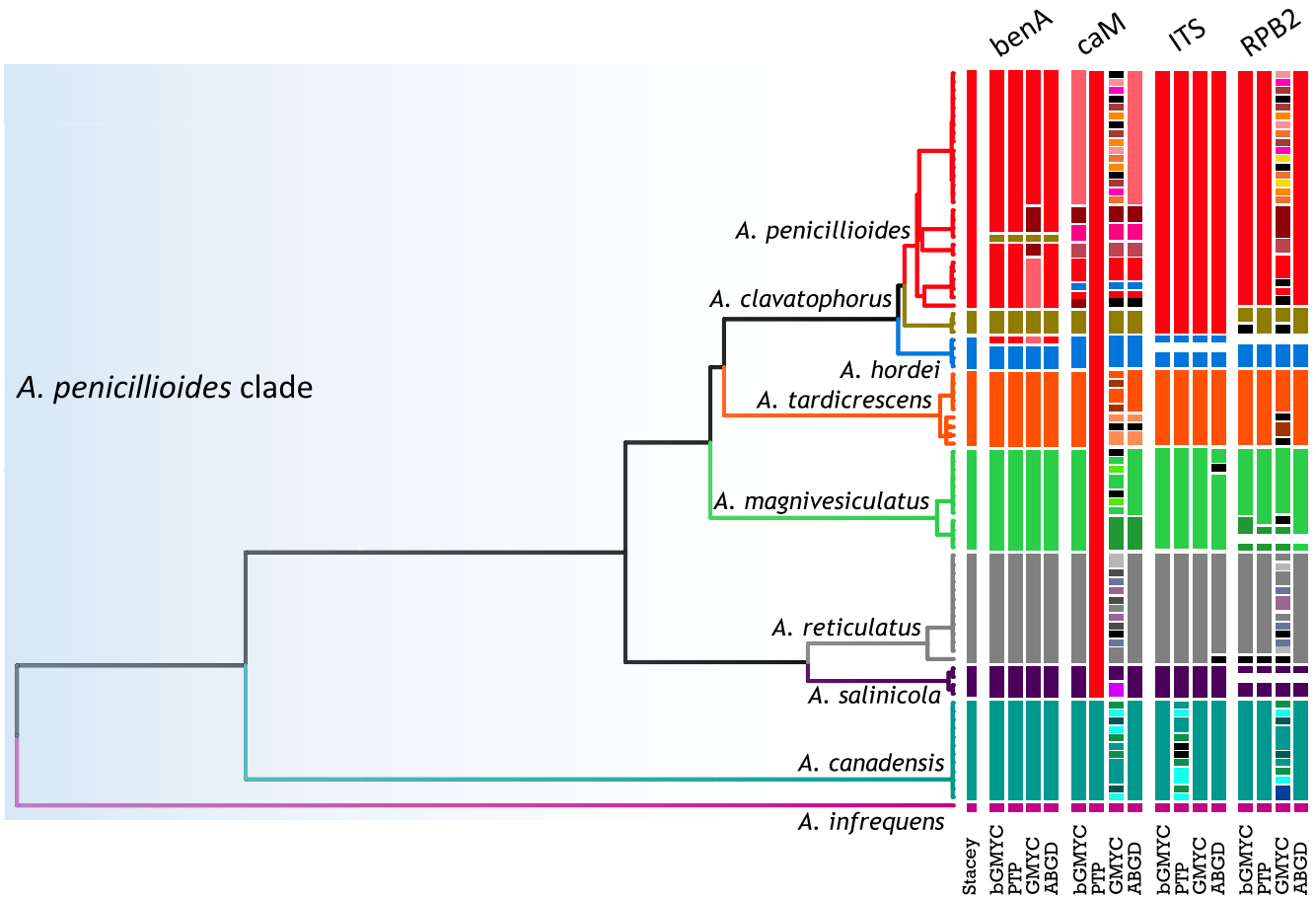


Fig. 4. Schematic representation of results of species delimitation methods in *A. penicillioides* clade (86 isolates). The results of multilocus method (STACEY) are compared to results of single-locus methods (bGMYC, PTP, GMYC, ABGD). Results from different methods are depicted with coloured bars highlighting congruence across methods. Displayed tree comes from STACEY analysis and is used only used solely for the comprehensive presentation of the results from different methods.

A. restrictus, *A. conicus*, *A. vitricola* and *A. penicillioides* clades are denoted in Fig. 5. We observed only slight differences in the topology of the species tree and statistical support of some nodes inferred with *BEAST when compared to the results of ML and BI analysis of the concatenated and partitioned dataset (see below). Several nodes had only limited support in all types of analyses and there is definitely some degree of uncertainty. These uncertainties can be visualized in DensiTree (Bouckaert 2010) (Fig. 6), that displays all trees created during the analysis except burn-in phase and trees with one of the three most common topologies are differently coloured. The most obvious problem is the position of *A. halophilicus*. Based on the available sequence data it is not clear whether it is situated basally within sect. *Restricti* or if it is rather phylogenetically related to some clade within the section. In addition, *BEAST analysis supported (PP = 1.00) an *A. conicus* clade consisting of six species, *A. conicus*, *A. domesticus*, *A. destruens*, *A. villosus, A. gracilis* and *A. pseudogracilis*, however this arrangement was not supported by the concatenated dataset analysis (see Fig. 7) where *A. gracilis* and *A. pseudogracilis* formed a distinct clade from the remaining four species. The position of *A. villosus* within the clade is also not fully resolved and differs between phylogenetic methods (compare Figs 5 and 7). The topology of the *A. penicillioides* clade was almost identical when using all methods. The exception is an unresolved position of *A. clavatophorus* and *A. tardicrescens*. The respective nodes gained only limited support while all other nodes are suported by PP = 1.00 in *BEAST analysis.

Bayesian and Maximum likelihood analysis of the partitioned sequence data

All species delimited by methods based on coalescent model were clearly supported by BI and ML analyses (Fig. 7). All clades representing species were supported by PP = 1.00 in BI analysis and at least 93 % bootstrap support in ML analysis. *Aspergillus halophilicus* was in a polytomy on the base of the section in agreement with the *BEAST analysis. Slight differences were observed in the interspecies relationships between core species of *A. penicillioides* as mentioned above. The ML and BI analysis of partitioned dataset did not separate *A. restrictus* and *A. conicus* clades as did the *BEAST analysis (compare Figs 5 and 7).

Comparisons with 454 data

A total of 1061 454-pyrosequences were found to belong to sect. *Restricti* and included in a dataset with 188 ITS barcodes generated during this study. The aligned dataset was 402 bp long and the calculated neighbour-joining tree is shown in Fig. 8. Many species were detected from dust samples, while *A. pseudogracilis, A. villosus, A. destruens, A. glabripes*, *A. salinicola* and *A. infrequens* were not detected. The phylogeny revealed 3–5 unnamed lineages from dust collected from Indonesia, Mexico, Micronesia, the Netherlands, South Africa, Thailand and Uruguay. Two singletons were also detected across the tree. Interestingly, each lineage is represented by

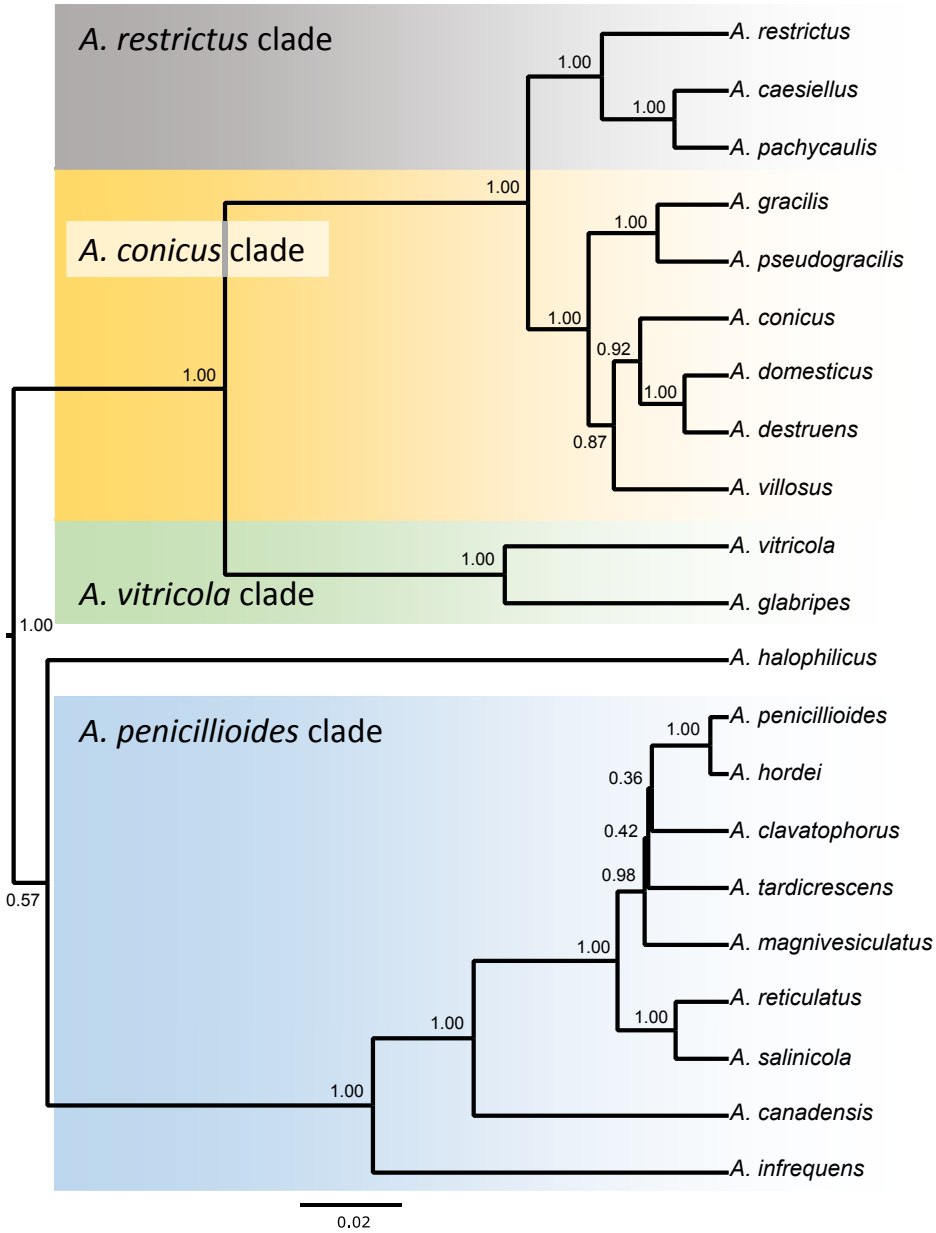


Fig. 5. Bayesian species tree based on sequence data from four loci of 193 isolates inferred by *BEAST with posterior probabilities appended to nodes. Terminal branches represent delimited species (each comprises all isolates of respective species).

OTU's from multiple countries, meaning they are probably common species.

Phenotype analysis

Micromorphology

Results of linear discriminant analysis (LDA) based on measurements of micromorphological characters (length and width of conidia, width of stipe and vesicle from the centre and edge of the colony and length of phialides) are shown on Figs 9–11. The values of individual phenotypic characters (average value ± standard deviation) for each species can be found in Table 6. The results of the analysis with individuals assigned to the species complexes are depicted on Fig. 9. While species from *A. penicillioides* complex and *A. restrictcus*/*conicus* complex form well-defined and relatively separated clusters, individuals from the *A. vitricola* complex formed two distinct clusters. These two clusters correspond to the two micromorphologically dissimilar species within the complex, i.e., *A. vitricola* and *A. glabripes*. It is also apparent from the analysis, that the most important character discriminating species complexes is the conidial size.

Figs 10, 11 show results of the analysis separately for particular species within species complexes/clades. The LDA clearly discriminated *A. pachycaulis* within the *A. restrictus* clade (Fig. 10A); the discrimation between *A. restrictus* and *A. caesiellus* was not so obvious because some isolates have similar micromorphologies. The discriminatory power of micromorphological characters within the *A. conicus* clade is low except for *A. gracilis* and *A. pseudogracilis* that were relatively well separated from other species (see Fig. 10C). The two species from the *A. vitricola* clade vary greatly in their micromorphological characters and consequently it resulted in clear separation of species in LDA.

The micromorphology of core species of the *A. penicillioides* clade is nearly identical, except *A. magnivesiculatus* that has much larger vesicles. It is more meaningful to compare micromorphology only in pairs of related species and even then

Table 5. Ensemble statistic (gsi_T) and p-value of the genealogical sorting index calculation.

Species	*benA*		*CaM*		ITS		*RPB2*		Consensus trees	
	gsi_T	p-value	gsi_T	p-value	gsi_T	p-value	gsi_T	p-value	gsi_T	p-value
***A. restrictus* clade**										
A. restrictus	0.997	0.0001	0.989	0.0001	0.898	0.0001	0.999	0.0001	0.983	0.0001
A. caesiellus	1	0.0001	0.959	0.0001	0.947	0.0001	0.959	0.0001	1	0.0001
A. pachycaulis	1	0.0003	1	0.0003	0.942	0.0001	1	0.0009	1	0.0005
***A. conicus* clade**										
A. conicus	0.999	0.0001	0.994	0.0001	0.914	0.0001	0.979	0.0001	1	0.0001
A. destruens	0.997	0.0001	1	0.0001	0.894	0.0001	0.991	0.0001	1	0.0001
A. domesticus	0.98	0.0001	0.897	0.0001	0.707	0.0001	0.891	0.0001	0.933	0.0001
A. gracilis	1	0.0001	1	0.0001	0.946	0.0001	1	0.0001	1	0.0001
A. villosus	1	0.0005	1	0.0007	0.952	0.0001	1	0.0006	1	0.0007
***A. vitricola* clade**										
A. vitricola	0.942	0.0001	1	0.0001	0.958	0.0001	0.995	0.0001	0.981	0.0001
A. glabripes	0.999	0.0001	1	0.0001	0.913	0.0001	0.979	0.0001	0.975	0.0001
***A. penicillioides* clade**										
A. penicillioides	0.871	0.0001	0.983	0.0001	0.948	0.0001	0.995	0.0001	0.960	0.0001
A. canadensis	0.987	0.0001	1	0.0001	1	0.0001	1	0.0001	1	0.0001
A. clavatophorus	1	0.0001	0.980	0.0001	1	0.0005	1	0.0001	1	0.0003
A. hordei	0.675	0.0001	1	0.0001	0.948	0.0001	0.997	0.0001	0.915	0.0001
A. magnivesiculatus	0.997	0.0001	0.974	0.0001	0.898	0.0001	0.998	0.0001	1	0.0001
A. reticulatus	0.995	0.0001	0.995	0.0001	0.930	0.0001	1	0.0001	0.977	0.0001
A. salinicola	0.971	0.0001	0.997	0.0001	0.987	0.0001	1	0.0003	1	0.0001
A. tardicrescens	1	0.0001	0.933	0.0001	1	0.0001	0.985	0.0001	1	0.0001

some species are not well separated as in the case of *A. penicillioides* and *A. hordei* (see Fig. 11C). On the other hand two pairs of closely related species, distant from the core group (*A. reticulatus* – *A. salinicola* and *A. canadensis* – *A. infrequens*) can be distinguished very well by the LDA (Fig. 11E, F).

Growth in an osmotic gradient

The ability to grow in an osmotic gradient proved to be very consistent during repeated testing of the same isolate and similarly, there was only low infraspecific variability. In general, it can be considered a useful supplementary taxonomic character that can distinguish some species. Growth curves created from measurements of the extension rate after 14 d are displayed on Figs 12–13. The *A. restrictus* clade contains less xerophilic species compared to others and grow faster on media with higher water activity (MEA or MEA + 5 % NaCl); *A. caesiellus* differs from the two remaining species of *A. restrictus* clade by faster growth on MEA + 10 % NaCl and 15 % NaCl. Species from the *A. conicus* clade show relatively similar growth curves; *A. domesticus*, *A. gracilis* and *A. pseudogracilis* grow slower than the three remaining species on MEA + 5 % and 10 % NaCl; *A. conicus* grows fastest on MEA + 5 % and 10 % NaCl; *A. villosus* grows faster on MEA and MEA + 5 % NaCl but with decreasing water activity the curve quickly declines and the species grows poorly on MEA + 15 % and 20 % NaCl. *Aspergillus vitricola* and *A. glabripes* differ significantly in their growth on MEA and MEA + 5 % NaCl.

Growth curves of species belonging to the core group of the *A. penicillioides* clade are nearly identical but differences can be found when comparing some species pairs (see Fig. 13), e.g. *A. penicillioides* and *A. tardicrescens* (Fig. 13B). *Aspergillus reticulatus* and *A. salinicola* grow faster than all other species on MEA + 5 % NaCl and slower on MEA + 20 % NaCl. *Aspergillus canadensis* and *A. infrequens* grow slowest on MEA + 5 % NaCl. The growth curves of the last mentioned species pairs are also different (see Fig. 13F).

Other phenotypic characters

Some other phenotypic characters are taxonomically relevant and useful for differentiation of species complexes or particular species. An overview of recorded characters is given in Tables 6 and 7 and their usability for differentiation of species complexes is detailed in the Taxonomy section; possibilities of distinguishing species within complexes are detailed in Distinguishing characters that follows the descriptions of species. The shape of conidial heads can be used to distinguish between clades. Conidial heads of *A. restrictus* clade form compact columns, heads of *A. conicus* clade are loosely columnar, those of *A. vitricola* clade are radiate and *A. penicillioides* clade have mainly globose conidial heads.

The extension rates of each species are given in Table 8. *Aspergillus pachycaulis* is the only member of the section *Restricti* that was able to grow on CY20S at 37 °C, but several species were able to grow at 37 °C on a more sucrose rich medium like M60Y. The extension rates of the species on optimal media (M40Y, M60Y, MEA + 10 % NaCl) was generally the same, but the species exhibit a greater degree of variability in more extreme conditions (high water activity, high temperature) and the ability or inability to grow on these suboptimal media can be used as

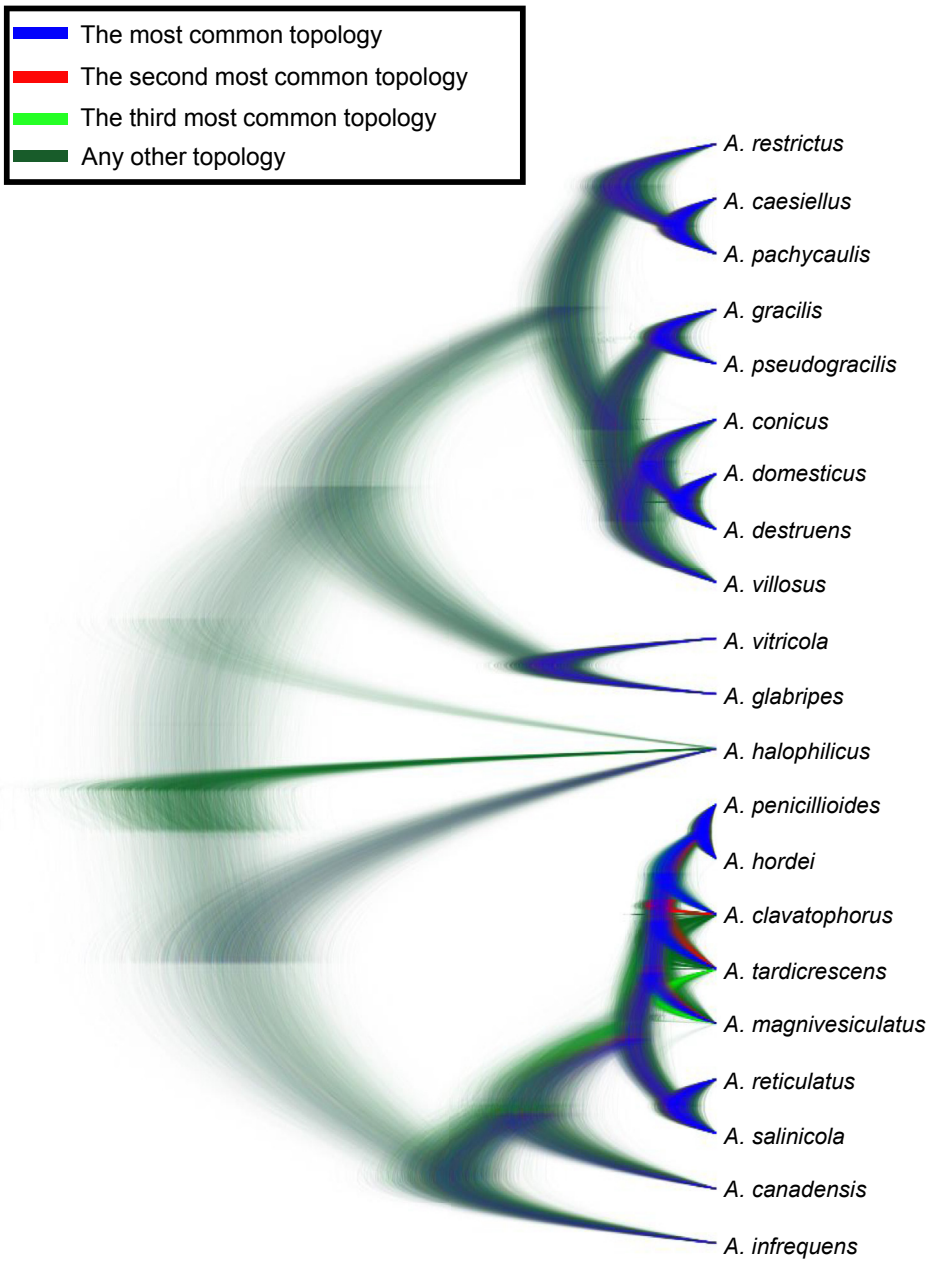


Fig. 6. Species tree inferred with *BEAST visualized by using DensiTree (Bouckaert 2010). All trees created in the analysis (except burn-in phase) are displayed. Trees with the most common topology are highlighted by blue colour, trees with the second most common topology by red colour, trees with the third most common topology by pale green and all other trees by dark green.

taxonomic characters to distinguish between closely related species (e.g. *A. conicus*, *A. domesticus* and *A. destruens*).

Exometabolite analysis

The overview of metabolites produced by sect. *Restricti* species is given in Table 9 and their absorption maxima and bracketed retention indexes are listed in Table S1. Six species had isolates that could produce mycophenolic acid: *A. glabripes*, *A. gracilis*, *A. halophilicus*, *A. pachycaulis*, *A. penicillioides* and *A. tardicrescens* (Table 9). We detected asperglaucide, its precursor aurantiamide and/or asperphenamate in all species in sect. *Restricti* (Table 9). Other extrolites detected include antarone A, asperentins, clavatols, cyclopaldic acid, orthosporins, naphtho-gamma-pyrones and chrysogine. Several other extrolites were detected including indole alkaloids, phthalides, pyrones and some compounds with yellow chromophores (Table 9). Members of the echinulin/neoechinulin/isoechinulin/cristatine biosynthetic family were detected in *A. halophilicus*, *A. hordei*, *A. magnivesiculatus*, *A. penicillioides* and *A. tardicrescens*, indicating a strong chemical relationship with *Aspergillus* section *Aspergillus*. However, while nearly all isolates in species of section *Aspergillus* produce the yellow auroglaucin and flaviglaucin-type metabolites (colouring the ascomata), these yellow metabolites were not found in any species of section *Restricti*.

TAXONOMY

Aspergillus* section *Restricti (Gams *et al.* 1985), Adv. *Penicillium Aspergillus* Syst.: 56.

Typus: *Aspergillus restrictus* G. Sm., J. Textile Inst. 22: T115. 1931.

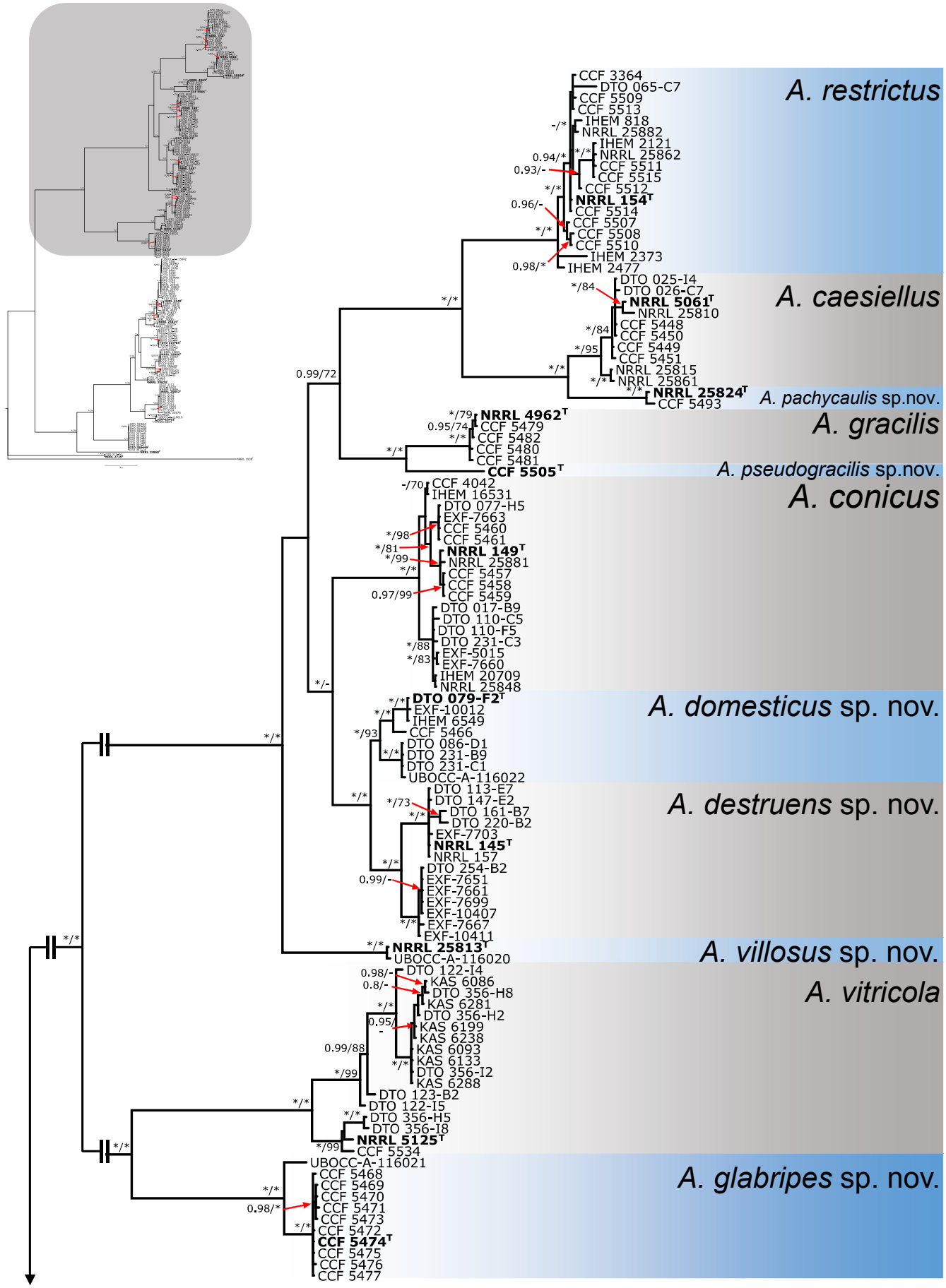


Fig. 7. Bayesian tree depicting the relationship of species from *Aspergillus* section *Restricti* based on four loci (partitioning scheme and substitution models are listed in Table 3). Total length of the concatenated alignment was 2093 characters, with 837 variable and 668 parsimony informative sites. Support values represent Bayesian posterior probability/maximum likelihood bootstrap values, 100 % bootstrap values and 1.00 posterior probability are designated by asterisk *. The ex-type isolates are designated by bold font and superscript T. *Hamigera avellanea* (NRRL 1938) was used as outgroup.

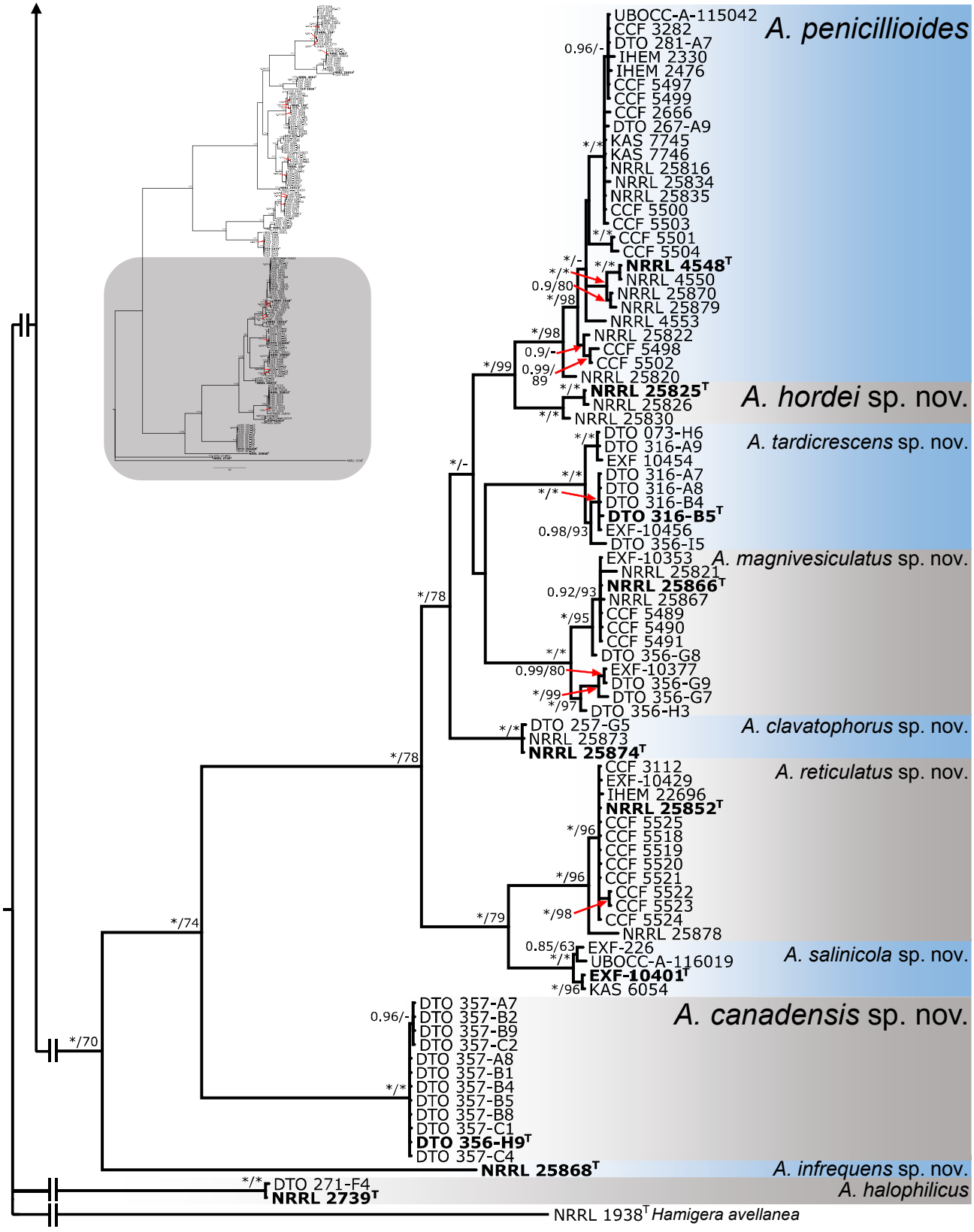


Fig. 7. (Continued).

The section includes 20 anamorphic species and one homothallic species. Xerophilic, growing optimally either on substrates containing high concentrations of sugar or salt or with low water content. Young conidial heads columnar (*A. restrictus* and *A. conicus* clade), radiate (*A. vitricola* clade) or globose (*A. penicillioides* clade). Conidiophores uniseriate with hyaline stipes, usually villose in SEM. Vesicles pyriform, subglobose or clavate, fertile over one to two thirds of the surface. Phialides flask-shaped, usually villose in SEM. Conidia ellipsoidal to ovate, barrel-shaped or subglobose, rough-walled; aculeate, tuberculate or

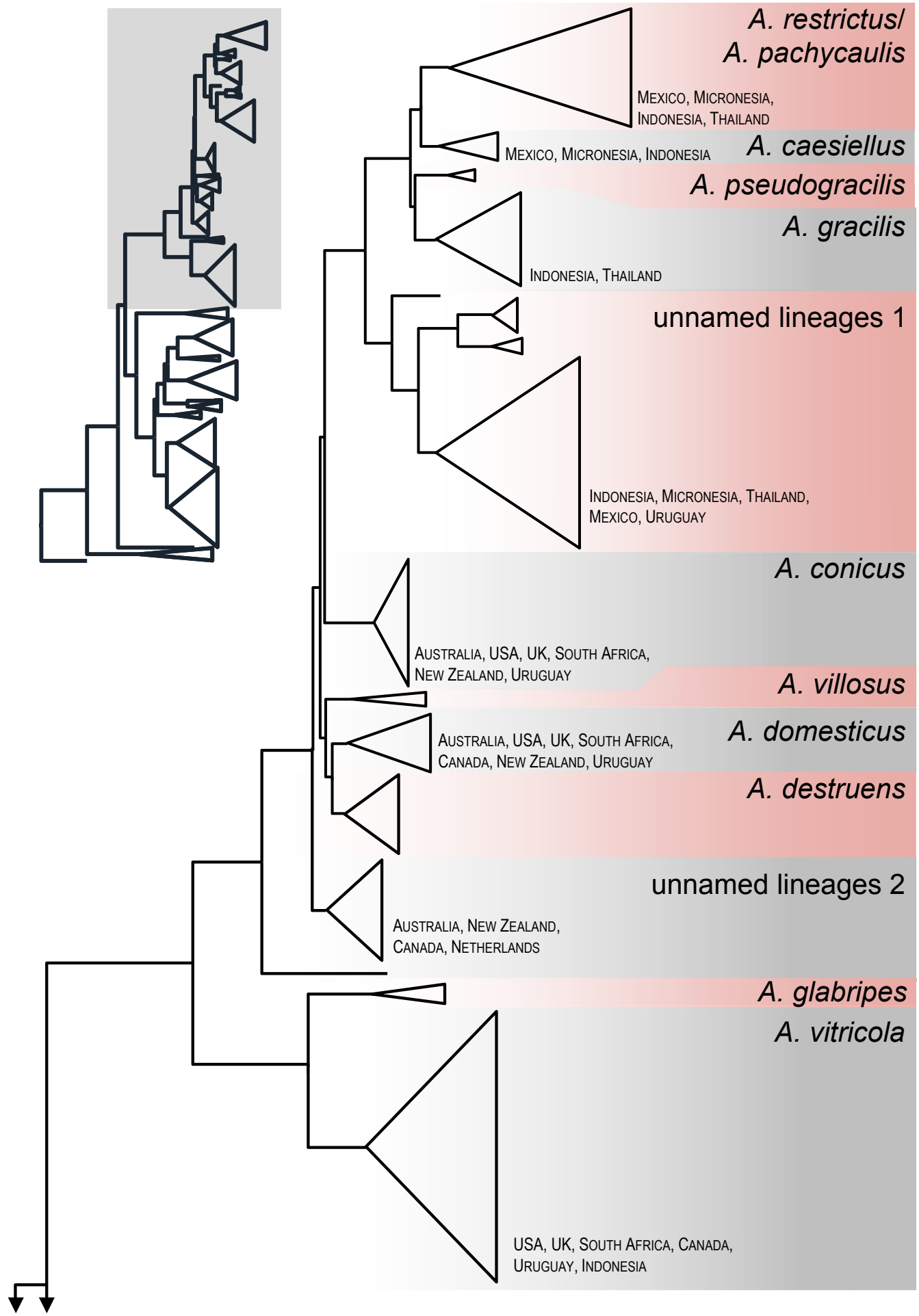


Fig. 8. Neighbour Joining tree created in MEGA 7 with 1000 bootstrap replicates based on ITS from 454-pyrosequences obtained from house dust samples (n = 1061; Amend *et al.* 2010) and from this study (n = 188). The tree is rooted with *Hamigera avellanea*. Monophyletic groups/species are collapsed and shown as proportional triangles. The information about country of origin pertains to and is given only for species found in house dust.

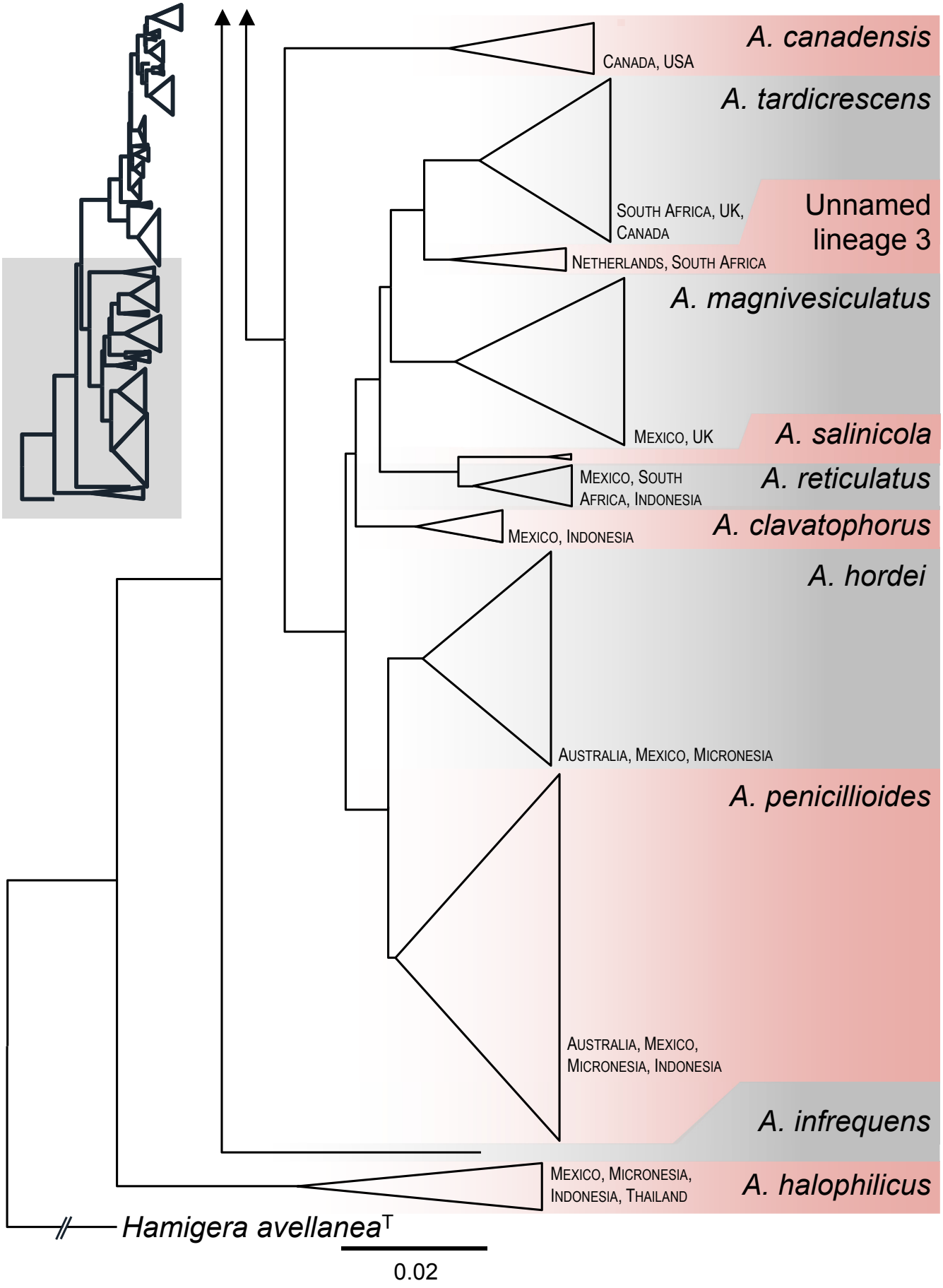


Fig. 8. (Continued).

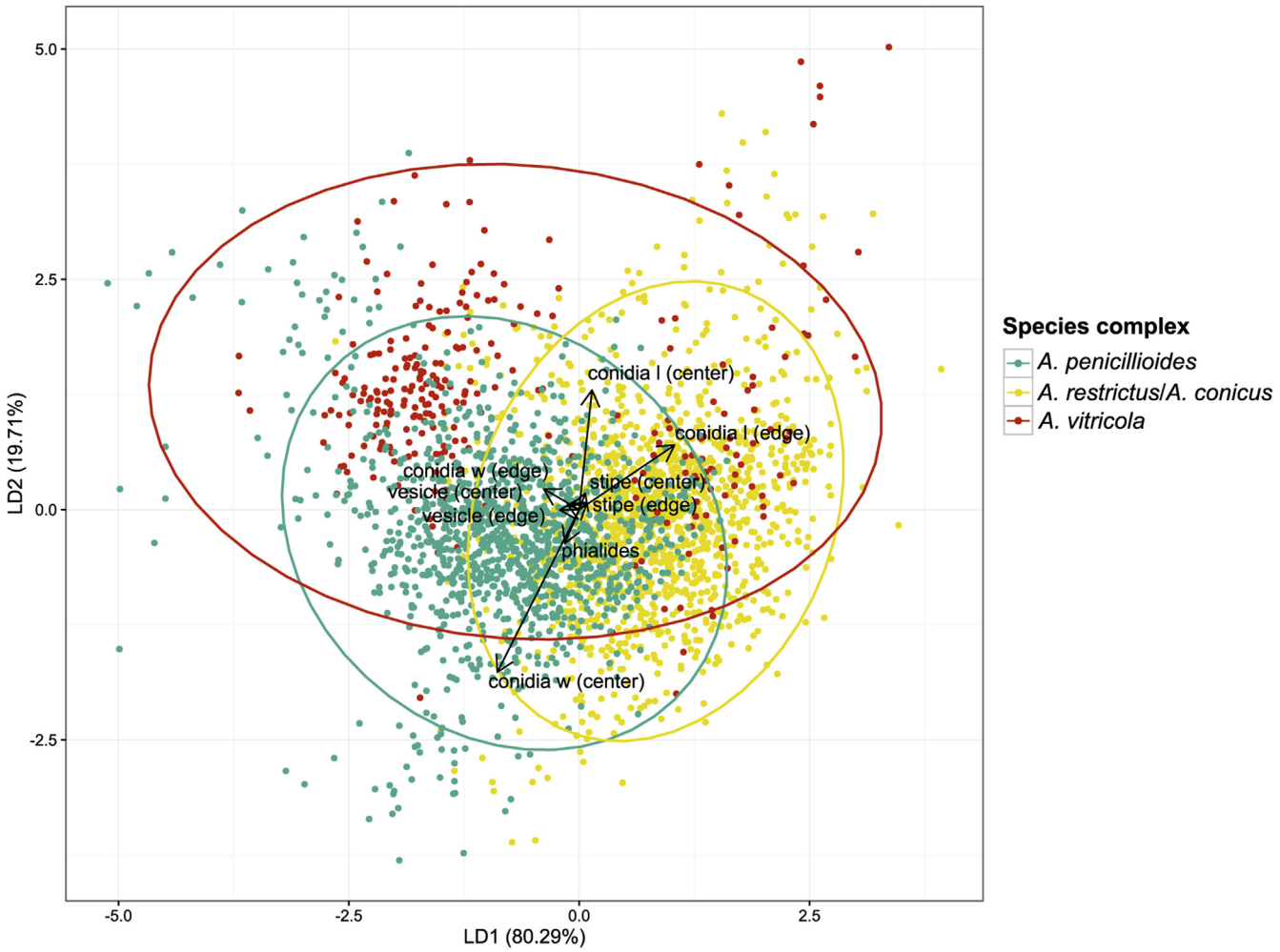


Fig. 9. Results of linear discriminant analysis (LDA) performed in R 3.3.1 based on micromorphological measurements of all individuals assigned into species complexes. Ellipses represent 95 % confidence interval and arrows represent the contribution of each character to the axes. The length (l) and width (w) of conidia, diameter of stipes and vesicles were measured separately from the center and the edge of colonies.

lobate-reticulate in SEM. Ascomata present only in *A. halophilicus*, cleistothecial, hyaline to pale yellow, globose to subglobose. Asci globose to subglobose. Ascospores hyaline, lenticular with two equatorial crests, convex surface finely roughened in the area neighbouring the equatorial region, dimpled in SEM.

Aspergillus restrictus clade

Aspergillus restrictus, A. caesiellus and A. pachycaulis are distinguished from all other members of sect. *Restricti* by compact columnar conidial heads, different growth parameters in an osmotic gradient (compared to other clades, they grow faster on media with high water activity, e.g. MEA and MEA + 5 % NaCl and slower on MEA + 20 % NaCl and MEA + 25 % NaCl) and rapid growth and deep green sporulation on CY20S.

Accepted species:

Aspergillus caesiellus Saito, J. Fac. Sci. Coll. Imper. Univ. Tokyo 18: 49. 1904. [MB205025].

Aspergillus pachycaulis F. Sklenar, S.W. Peterson, Ž. Jurjević & Hubka, this study. [MB823048].

Aspergillus restrictus G. Sm., J. Textile Inst. 22: T115. 1931. [MB276290].

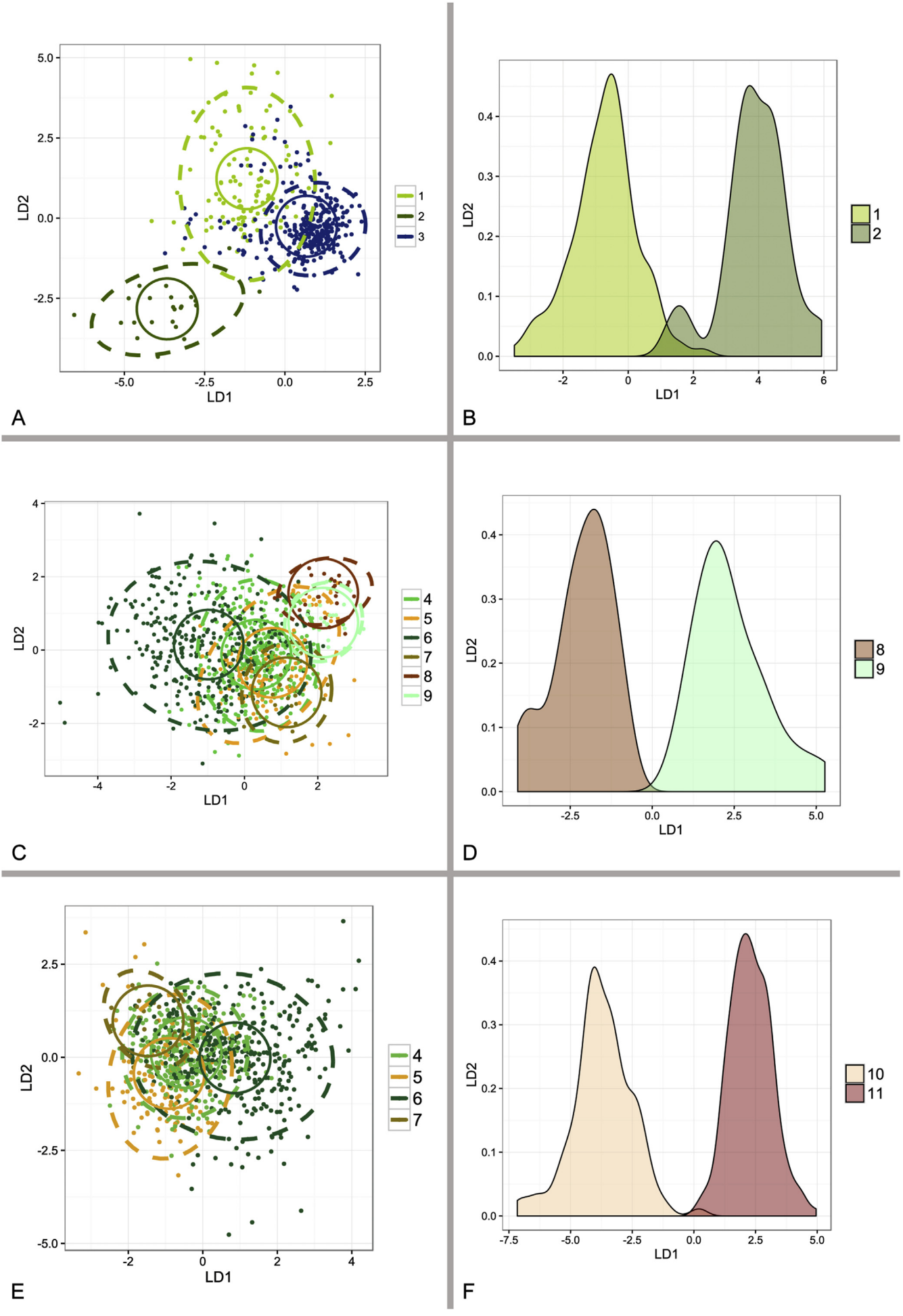
Aspergillus conicus clade

This clade contains six species (*A. conicus*, *A. domesticus*, *A. destruens*, *A. villosus*, *A. gracilis* and *A. pseudogracilis*). Members of the *A. conicus* clade have loosely columnar conidial heads, while species from the closely related *A. vitricola* clade have radiate heads. Several characters are useful for distinguishing between members of the *A. conicus* and *A. penicillioides* clade. The colour of sporulation of *A. conicus* clade species is usually paler green, colony reverse colour on M40Y is usually olive grey compared to the brown or red reverse of *A. penicillioides* clade species. Further, the members of *A. penicillioides* clade produce white cottony secondary mycelium on M60Y in contrast to *A. conicus* clade and conidial heads are usually globose. Conidia of *A. conicus* clade species in SEM are usually aculeate, whereas conidia of *A. penicillioides* clade species are tuberculate or lobate-reticulate. The differences from *A. restrictus* clade were described above (see distinguishing characters of *A. restrictus*).

Accepted species:

Aspergillus conicus Blochwitz in Dale, Ann. Mycol. 12: 38. 1914. [MB120214].

Aspergillus destruens Zalar, F. Sklenar, S.W. Peterson & Hubka, this study. [MB818930].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D
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E
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F
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LD1
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11

Aspergillus domesticus F. Sklenar, Houbraken, Zalar & Hubka, this study. [MB818931].
Aspergillus gracilis Bainier, Bull. Soc. Mycol. Fr. 23: 92. 1907. [MB167554].
Aspergillus pseudogracilis F. Sklenar, Ž. Jurjević & Hubka, this study. [MB818932].
Aspergillus villosus F. Sklenar, S.W. Peterson & Hubka, this study. [MB818933].

Aspergillus vitricola clade

This clade contains two species, *A. vitricola* and *A. glabripes*, and is defined by their radiate conidial heads, smooth stipes and phialides in SEM (unique through the whole section), and aculeate conidia in SEM.

Accepted species:
Aspergillus vitricola Ohtsuki, Bot. Mag. (Tokyo) 75: 436. 1962. [MB326665].
Aspergillus glabripes F. Sklenar, Ž. Jurjević & Hubka, this study. [MB818934].

Aspergillus penicillioides clade

This clade encompasses nine species (Fig. 7). *Aspegillus tardicrescens*, *A. clavatophorus*, *A. magnivesiculatus* and *A. hordei* are closely related to *A. penicillioides*, while the remaining four species are relatively distant. The core species can be considered the most xerophilic members of the section, and generally some of the most xerophilic fungi. Conidial heads of these species are always globose when young and sporulation colour is usually darker compared to species from other clades. Stipes are covered by short hairs observed with SEM and conidia are usually born as ellipsoidal (not cylindrical) and have tuberculate or lobate-reticulate ornamentation in SEM.

Accepted species:
Aspergillus canadensis Visagie, Yilmaz, F. Sklenar & Seifert, this study. [MB818935].
Aspergillus clavatophorus F. Sklenar, S.W. Peterson & Hubka, this study. [MB818936].
Aspergillus hordei F. Sklenar, S.W. Peterson & Hubka, this study. [MB818937].
Aspergillus infrequens F. Sklenar, S.W. Peterson & Hubka, this study. [MB818938].
Aspergillus magnivesiculatus F. Sklenar, Zalar, Ž. Jurjević & Hubka, this study. [MB818939].
Aspergillus reticulatus F. Sklenar, Ž. Jurjević, S.W. Peterson & Hubka, this study. [MB818940].
Aspergillus salinicola Zalar, F. Sklenar, Visagie & Hubka, this study. [MB818941].
Aspergillus tardicrescens F. Sklenar, Houbraken, Zalar & Hubka, this study. [MB818942].
Aspergillus penicillioides Speg., Revista Fac. Agron. Univ. Nac. La Plata 2: 245. 1896. [MB309234].

Aspergillus halophilicus clade

This clade contains only one species, *Aspergillus halophilicus*. It is easily recognisable, because it is the only species from the section that produces sexual reproductive state.

Accepted species:
Aspergillus halophilicus C.M. Chr., Papav. & C.R. Benj., Mycologia 51: 636. 1959. [MB326633].

Species descriptions

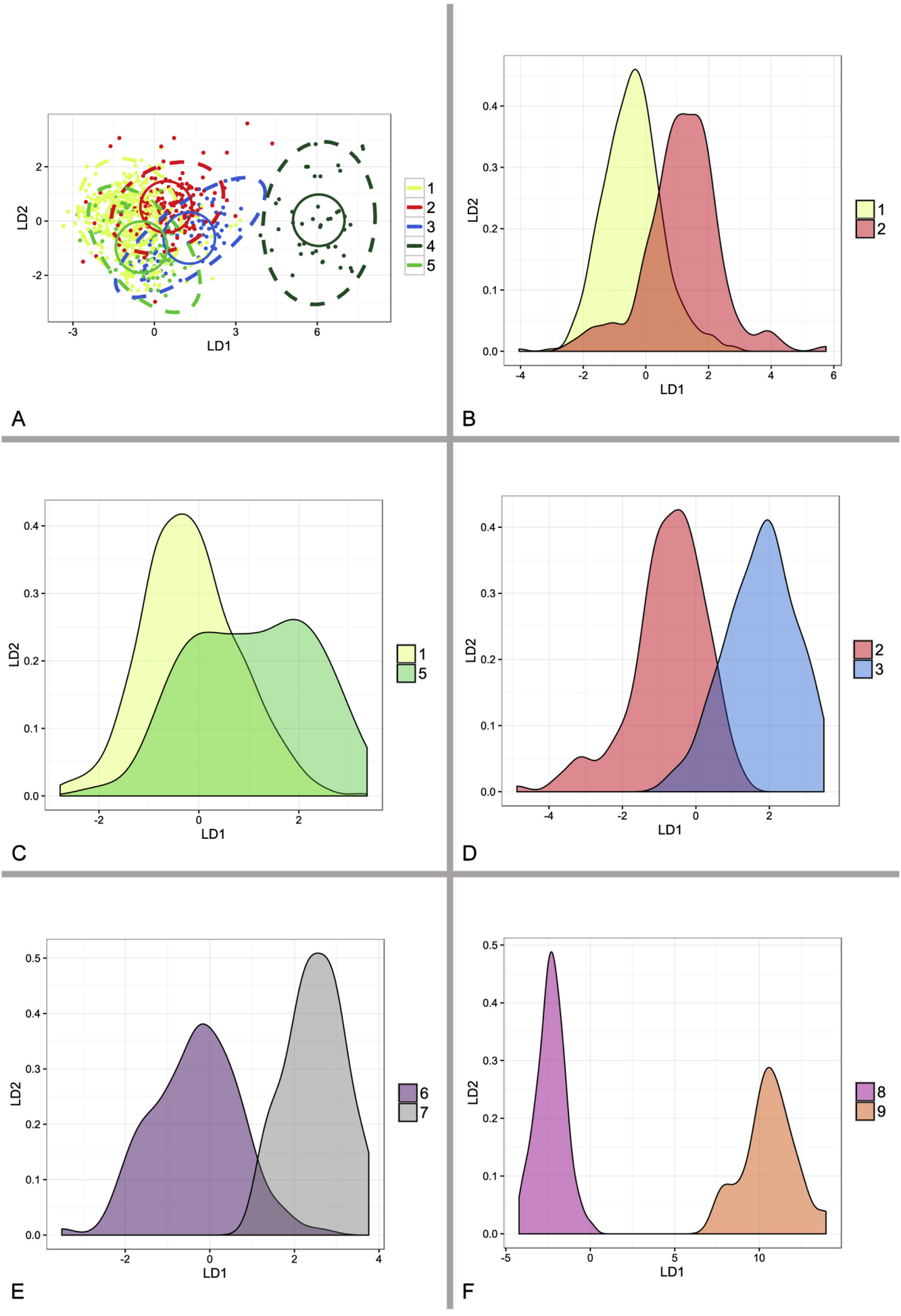
Aspergillus caesiellus Saito, J. Fac. Sci. Coll. Imper. Univ. Tokyo 18: 49. 1904, emended description. MycoBank MB205025. Fig. 14.
Synonyms: *Aspergillus gracilis* var. *sartoryi* Bat., O.G. Lima & A.F. Vital, Mycopath. Mycol. Appl. 8: 96. 1957.
Aspergillus restrictus mut. *eborinus* G. Sm., Trans. Brit. Mycol. Soc. 44: 45. 1961.

Typus: **Japan**, unknown substrate, unknown year of isolation, isolated by K. Saito [neotype Herb. IMI 172278 designated by Samson & Gams (1985) and modified by Pitt & Samson (1993), culture ex-type NRRL 5061 = CBS 470.65 = DTO 093-H3 = ATCC 11905 = IMI 172278 = CCF 5447 = IBT 34620].

Colony diam, 14 d (*mm*): M40Y 58–60; CYA 11–22; CY20S 21–35; CY20S 30 °C 12–40; CY20S 37 °C No growth; DG18 32–38; MEA 17–22; M60Y 48–60; M60Y 30 °C 45–60; M60Y 37 °C 32–55; MEA + 10 % NaCl 36–45.

Colony characters: M40Y 25 °C, 14 d: flat; colony surface floccose; margin entire; mycelial areas white; sporulation greyish turquoise (24E4); soluble pigment absent; exudate absent; reverse centrally sunburn light brown (6D5) to greyish orange (5B4) in margins. CYA 25 °C, 14 d: with central depression; colony surface floccose, margin entire; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally dull blue (23E4) to orange white (5A2) in margins. CY20S 25 °C, 14 d: raised; colony surface floccose; margin entire to undulate; mycelial areas white; sporulation jungle green (25F5) to dull green (25E4); soluble pigment absent; exudate absent; reverse centrally baby blue (bluish grey) (23B3) to orange grey (5B2) in margins. DG18 25 °C, 14 d: centrally raised; colony surface velvety to floccose in the centre, margin entire to delicately filiform; mycelial areas white; sporulation dull green (25D4); soluble pigment absent; exudate absent; reverse centrally birch grey (5C2) to orange grey (5B2) in margins. MEA 25 °C, 14 d: with central depression, irregulary wrinkled; colony surface floccose, margin delicately undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally dark blonde (5D4) to greyish orange (5B3) in margins. M60Y 25 °C,

Fig. 10. Results of linear discriminant analysis (LDA) performed in R 3.3.1 based on micromorphological measurements of species from *A. restrictus*, *A. conicus* and *A. vitricola* clade. Dashed ellipses represent 95 % confidence interval, full circles euclidean distances. Analyses involving only two species (B, D, F) are represented by probability density function. 1 – *A. restrictus*; 2 – *A. caesiellus*; 3 – *A. pachycaulis*; 4 – *A. conicus*; 5 – *A. domesticus*; 6 – *A. destruens*; 7 – *A. villosus*; 8 – *A. gracilis*; 9 – *A. pseudogracilis*; 10 – *A. vitricola*; 11 – *A. glabripes*.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14 d: flat; colony surface filamentous; margin filiform; mycelial areas white; sporulation dull green (27E3); soluble pigment absent; exudate absent; reverse pale yellow (3A3). MEA + 10 % NaCl 25 °C, 14 d: centrally raised; colony surface floccose; margin irregular; mycelial areas white; sporulation dull green (26D3); soluble pigment absent; exudate absent; reverse centrally light yellow (4A4) to pastel yellow (3A4) in margins.

Micromorphology: Conidial heads columnar, frequently sinuous. Conidiophores uniseriate. Stipes smooth, densely covered by long hairs in SEM, occasionally with septa, 5–7.5 µm wide. Vesicles pyriform to clavate, 11–16 µm in diameter. Phialides flask-shaped, densely covered by long hairs in SEM, 8–9 µm long, covering one third to one half of the vesicle. Conidia borne as smooth cylinders, at maturity ellipsoidal to ovate, connectives evident, echinulate, aculeate in SEM, 4.5–6 × 3–4.5 µm.

Distinguishing characters: See distinguishing characters of *A. restrictus*. The stipes of *A. caesiellus* are usually wider than those of *A. restrictus* and narrower than those of *A. pachycaulis*, vesicles of *A. caesiellus* are usually larger than those of *A. restrictus* and smaller than those of *A. pachycaulis*.

Taxonomic notes: The LSU region of the ex-type strain of *A. gracilis* var. *sartoryi* (NRRL 4745; GenBank U29642) is identical with the ex-type strain of *A. caesiellus* and confirms conclusions of Raper and Fennell (1965) based on morphology. Authentic strain of *A. restrictus* mut. *eborinus* NRRL 4756 is conspecific with *A. caesiellus* based on molecular data.

Additional materials examined: **Germany**, air, unknown year of isolation, collector unknown, DTO 077-H6 = CBS 117330. **Germany**, indoor environment, 2006, collector unknown, DTO 026-C7, DTO 025-I4 = IBT 34538. **India**, Gorakpur, unknown substrate, before 1974, isolated by T. N. Bhargana, NRRL 25861. **Indonesia**, corn kernels, unknown year of isolation, collector unknown, DTO 065-D7. **Panama**, cloth, 1945, isolated by W. H. Weston, NRRL 25810 = CCF 5662. **The Netherlands**, wood of crate, imported from China, unknown year of isolation, isolated by J. Houbraken, DTO 060-H9. **USA**, Delaware, outside air, 2012, isolated by Ž. Jurjević, CCF 5450 = EMSL No. 1614. **USA**, Pennsylvania, home air, 2010, isolated by Ž. Jurjević, CCF 5448 = EMSL No. 1383 = IBT 34621. **USA**, Delaware, air, pineapple room, warehouse, 2012, isolated by Ž. Jurjević, CCF 5451 = EMSL No. 1650 = IBT 34622. **USA**, Delaware, home air, 2011, isolated by Ž. Jurjević, CCF 5449 = EMSL No. 1499. **USA**, Florida, hobnail shoes, 1944, isolated by W. H. Weston, NRRL 25815 = CCF 5663 = DTO 356-D1.

Aspergillus canadensis Visagie, Yilmaz, F. Sklenar & Seifert, sp. nov. Mycobank MB818935. Fig. 15.

Etymology: Named after country of origin.

Typus: **Canada**, Nova Scotia, Wolfville, house dust, 2015, collected by A. Walker, isolated by C.M. Visagie, (holotype DAOM 740109, culture ex-type CCF 5548 = KAS 6194 = DTO 356-H9 = IBT 34520 = IBT 34642 = NRRL 66614).

Colony diam, 14 d (*mm*): M40Y 25–29; CYA No growth; CY20S (0)10–11; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 7–15; MEA No growth; M60Y 23–42; M60Y 30 °C 2–20; M60Y 37 °C No growth; MEA + 10 % NaCl 17–33.

Colony characters: M40Y 25 °C, 14 d: Colonies umbonate, irregularly wrinkled, surface velutinous; margin delicately filiform; mycelial areas white; sporulation jungle green (25F5) to pale turquoise (24A3); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2), corn greyish yellow (4B5) to champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: no growth. CY20S 25 °C, 14 d: cerebriform, wrinkled; colony surface velutinous, margin undulate; mycelial areas white; sporulation pale turquoise (24A3); soluble pigment absent; exudate absent; reverse centrally dark green (27F7) to greenish grey (25D2), orange white (5A2) in margins. DG18 25 °C, 14 d: centrally raised, radially wrinkled, colony surface velutinous, margin undulate; mycelial areas white; sporulation turquoise grey (24B2); soluble pigment absent; exudate absent; reverse centrally orange grey (5B2) to orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: umbonate; colony surface floccose; margin delicately filiform; mycelial areas white; sporulation greyish green (25C5); soluble pigment absent; exudate absent; reverse centrally olive green (2F8) to mustard yellow (3B6), yellowish white (4A2) in the margins. MEA + 10 % NaCl 25 °C, 14 d: flat to umbonate, irregularly wrinkled; colony surface velutinous; margin entire to delicately filiform; mycelial areas white; sporulation dull green (25D4) to greyish green (25B4); soluble pigment absent; exudate absent; reverse centrally brownish grey (5F2) to mouse grey (5E3), champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads radiate. Conidiophores uniseriate. Stipes smooth, sparsely covered by bundles of long hairs in SEM, 3.5–5 µm wide in the middle part, non-septate. Vesicles pyriform to clavate, (9–)13–16(–20) µm wide. Phialides flask-shaped, sparsely covered by long hairs in SEM, 6–8 µm long, covering two thirds of the vesicle. Conidia borne smooth and ellipsoidal, becoming rough-walled, subglobose or barrel-shaped at maturity, lobate-reticulate in SEM, commonly remaining in chains, connectives evident, 3–4 × 2.5–3 µm.

Distinguishing characters: *Aspergillus canadensis* and *A. infrequens* are phylogenetically distinct from the core species in the *A. penicillioides* clade. *Aspergillus canadensis* is unable to grow on CYA and MEA at 25 °C and grows much slower on M60Y at 30 °C than remaining species from the *A. penicillioides* clade. The phialides of this species are the shortest across the whole section.

Additional materials examined: **Canada**, Ontario, Ottawa, house dust, 2015, collected by J. Mack, isolated by C.M. Visagie, DAOMC 251505 = KAS 6095, DAOMC 251506 = KAS 6269, KAS 7704 = DTO 357-A7, KAS 7705 = DTO 357-A8, CCF 5549 = KAS 7706 = DTO 357-A9 = IBT 34639 = NRRL 66615, KAS 7707 = DTO 357-B1, CCF 5550 = KAS 7708 = DTO 357-B2 = IBT 34637, CCF 5551 = KAS 7709 = DTO 357-B3 = IBT 34640, CCF 5552 = KAS 7710 = DTO 357-B4 = IBT 34636, KAS 7711 = DTO 357-B5, KAS 7716 = DTO 357-B8, KAS 7717 = DTO 357-B9, KAS 7718 = DTO 357-C1, CCF 5553 = KAS 7719 = DTO 357-C2 = IBT 34638, KAS 7720 = DTO 357-C3 = IBT 34641, KAS 7721 = DTO 357-C4.

Note: The majority of strains of *A. canadensis* quickly degenerated in culture and changed significantly their phenotype (restricted growth, waxy colonies with poor or no sporulation).

Aspergillus clavatophorus F. Sklenar, S.W. Peterson & Hubka, sp. nov. MycoBank MB818936. Fig. 16.

Etymology: Refers to the clavate vesicles of the conidiophores

Fig. 11. Results of linear discriminant analysis (LDA) performed in R 3.3.1 based on micromorphological measurements of species from *A. penicillioides* clade. Dashed ellipses represent 95 % confidence interval, full circles euclidean distances. Analyses involving only two species (B, D, F) are represented by probability density function. 1 – *A. penicillioides*; 2 – *A. tardicrescens*; 3 – *A. clavatophorus*; 4 – *A. magnivesiculatus*; 5 – *A. hordei*; 6 – *A. reticulatus*; 7 – *A. salinicola*; 8 – *A. canadensis*; 9 – *A. infrequens*.

Table 6. Overview of conidia and conidiophore characteristics.

Species	Conidia				Conidiophores				
	Length	Width	Ornamentation (SEM)	Mature conidia shape	Stipe width (µm)	Vesicle width (µm)	Phialides length (µm)	Stipe surface (SEM)	Shape of vesicle
***A. restrictus* clade**									
A. restrictus	3.5–4.5 (4.1±0.4)	2.5–3 (2.9±0.4)	Aculeate	Ellipsoidal to ovate	4–5.5 (4.8±1)	9–12 (11.5±2)	8–9 (8.4±0.9)	Densely covered by long hairs	Pyriform to clavate
A. caesiellus	4.5–6 (5±0.8)	3–4.5 (3.6±0.5)	Aculeate	Ellipsoidal to ovate	5–7.5 (5.5±0.8)	11–16 (13.2±2)	8–9 (8.2±2)	Densely covered by long hairs	Pyriform to clavate
A. pachycaulis	4–5 (4.4±0.2)	2.5–3.5 (3±0.2)	Aculeate	Ellipsoidal to ovate	7–8 (7.3±0.8)	16–20 (18.6±1.8)	8–9 (8.7±0.5)	Densely covered by short hairs	Pyriform to subclavate
***A. conicus* clade**									
A. conicus	4–4.5 (4.1±0.4)	2.5–3 (2.9±0.3)	Aculeate	Globose to subglobose or barrel-shaped	4–5.5 (4.5±0.9)	8–13 (10.5±2.5)	8.5–10 (9.2±1.5)	Sparsely covered by short hairs	Pyriform, spatulate to clavate
A. destruens	3.5–5.5 (4.3±0.6)	2.5–3.5 (3.1±0.4)	Aculeate	Subglobose to ovate	4–6 (4.2±0.9)	8–14 (10.2±2.1)	8.5–11.5 (8.6±1.7)	Densely covered by long hairs	Predominantly ellipsoidal to pyriform
A. domesticus	3.5–4.5 (4.2±0.5)	2.5–3.5 (2.8±0.3)	Aculeate	Ellipsoidal or barrel-shaped	4–6 (4.8±0.8)	7–13 (10.6±2.7)	8.5–11 (9.7±1)	Densely covered by long hairs	Pyriform, spatulate to clavate
A. gracilis	3–4 (3.4±0.2)	2–3 (2.5±0.2)	Aculeate	Subglobose or barrel-shaped	3–5 (4.1±0.5)	6.5–9 (8±0.7)	7.5–9 (8.7±0.6)	Densely covered by very long hairs	Spatulate to clavate
A. pseudogracilis	3–4 (3.6±0.3)	2–3 (2.7±0.2)	Aculeate	Subglobose or barrel-shaped	4–6 (5±0.6)	9–13 (10.3±1.6)	9–10.5 (10±0.4)	Densely covered by long hairs	Spatulate to clavate
A. villosus	3.5–4.5 (3.9±0.4)	2.5–3.5 (2.9±0.2)	Tuberculate	Ellipsoidal or barrel-shaped	5–6 (5.5±0.5)	10–14 (12.6±1.4)	9–12 (10.8±1.1)	Densely covered by long hairs	Pyriform, spatulate to clavate
***A. vitricola* clade**									
A. vitricola	4.5–5.5 (4.8±0.4)	3–4 (3.4±0.3)	Aculeate	Subglobose to ellipsoidal	4.5–6 (5.1±0.6)	7–12 (9.6±1.7)	8–10 (9.1±0.7)	Smooth	Spatulate, pyriform to clavate
A. glabripes	3.5–4.5 (4.1±0.2)	2–3 (2.9±0.2)	Aculeate	Subglobose	6–8 (6.9±0.8)	16–22 (19.9±2.3)	7–9 (8.5±1)	Smooth	Globose, pyriform to subclavate
***A. penicillioides* clade**									
A. penicillioides	3.5–4.5 (4±0.6)	2.5–3.5 (3±0.5)	Tuberculate	Subglobose or barrel-shaped	4–6 (5.1±0.9)	10–18 (13.5±2.3)	8–10 (9.3±1.7)	Sparsely covered by short hairs	Pyriform, spatulate to clavate
A. canadensis	3–4 (3.3±0.4)	2.5–3 (2.8±0.4)	Lobate-reticulate	Subglobose or barrel-shaped	3.5–5 (4.2±0.7)	(9–)13–16(–20) (13.9±2.5)	6–8 (6.9±0.4)	Sparsely covered by bundles of long hairs	Pyriform to clavate

A. clavatophorus	4–5 (4.8±0.2)	3–4 (3.5±0.2)	Tuberculate	Subglobose, ovate or barrel-shaped	6–9 (6.6±0.5)	14–19 (16.5±1.3)	8–10 (9.3±0.7)	Densely covered by short hairs	Spatulate to clavate
A. hordei	3.5–5 (4.1±0.4)	2.5–4 (3.2±0.4)	Lobate-reticulate	Globose or barrel-shaped	4.5–6 (5.5±0.6)	12–14.5 (13.2±1.4)	8.5–11 (9.5±1)	Densely covered by short hairs	Spatulate, ellipsoidal, pyriform to subclavate
A. infrequens	4–5 (4.8±0.3)	3–4 (3.6±0.3)	Aculeate	Subglobose, ovate or barrel-shaped	6–7 (6.7±0.8)	(10–)13–17(–20) (15.9±2.6)	8–10 (9.5±1)	Sparsely covered by bundles of long hairs	Pyriform, spatulate to clavate
A. magnivesiculatus	4.5–5.5 (5.2±0.3)	3.5–4.5 (3.9±0.3)	Tuberculate	Ovate to barrel-shaped	5–7 (5.9±1.2)	17–22 (21.4±3.2)	9–11 (9.6±0.8)	Densely covered by short hairs	Pyriform, subclavate to clavate
A. reticulatus	3.5–4.5 (3.9±0.3)	2.5–3.5 (3±0.3)	Tuberculate to lobate-reticulate	Globose or barrel-shaped	5–7 (6±1)	14–20 (16.3±3.2)	9–11 (9.3±0.8)	Densely covered by short hairs	Globose or pyriform
A. salinicola	3–4 (3.6±0.4)	2–3 (2.6±0.2)	Tuberculate to lobate-reticulate	Globose to subglobose	4–6 (3.6±0.4)	11–14 (12.6±1.3)	8–9 (8.7±0.4)	Sparsely covered by short hairs	Pyriform, spatulate to subclavate
A. tardicrescens	3.5–4.5 (4±0.4)	2.5–3.5 (2.9±0.3)	Tuberculate	Subglobose or barrel-shaped	5–8 (6±1.2)	14–19 (16.4±3.3)	8–11 (8.9±1.2)	Densely covered by short hairs	Subglobose, spatulate, pyriform to subclavate

Typus: **USA**, Georgia, Athens, mouldy paper, 1987, isolated by R. T. Hanlin (holotype PRM 944440, isotype PRM 944441, culture ex-type NRRL 25874 = CCF 5454 = IBT 34560 = IBT 34823 = DTO 356-D8).

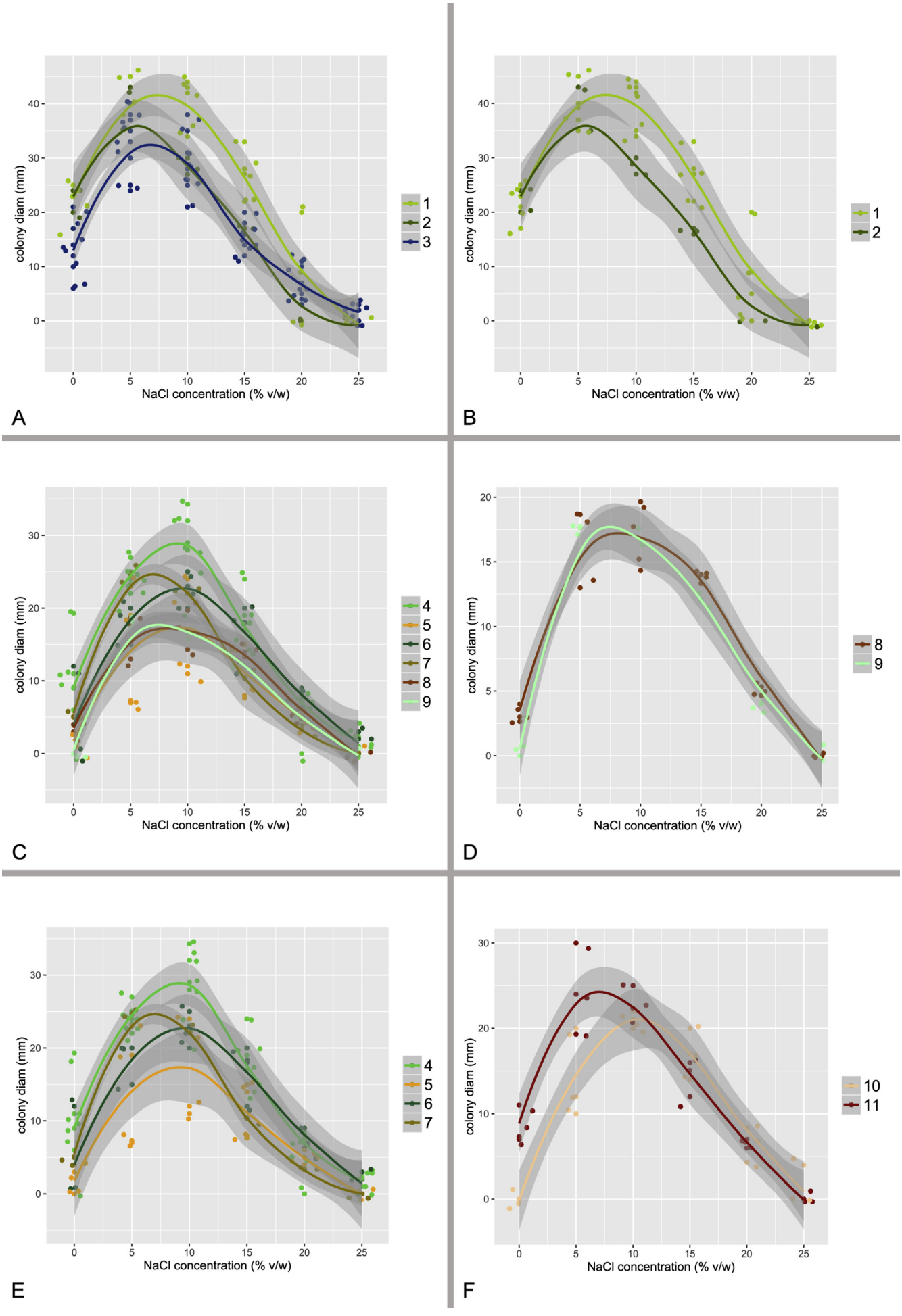
Colony diam, 14 d (*mm*): M40Y 27–33; CYA No growth; CY20S 7–16; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 18–25; MEA No growth; M60Y 35–44; M60Y 30 °C 29–54; M60Y 37 °C (0)5–17; MEA + 10 % NaCl 18–28.

Colony characters: M40Y 25 °C, 14 d: Colonies raised to umbonate, surface floccose to velutinous; margin entire to delicately filiform; mycelial areas white; sporulation greyish green (28D6); soluble pigment absent; exudate absent; reverse centrally coffee brown (5F7), golden brown (5D7) to pale yellow (4A3) in margins. CYA 25 °C, 14 d: no growth to microcolonies. CY20S 25 °C, 14 d: convex, radially wrinkled; colony surface floccose, margin undulate; mycelial areas white; sporulation rare, sand greyish yellow (4B3); soluble pigment absent; exudate absent; reverse centrally yellowish grey (4B2) to orange white (5A2) in margins. DG18 25 °C, 14 d: centrally raised; colony surface floccose to cottony, margin entire to delicately filiform; mycelial areas white; sporulation aquamarine greyish green (25B4) to pale green (27A3); soluble pigment absent; exudate absent; reverse centrally greyish turquoise (24C3) to orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: raised; colony surface floccose to lanose; margin entire to delicately filiform; mycelial areas white; sporulation greyish green (25D6); soluble pigment absent; exudate absent; reverse centrally dark brown (6F6) to golden brownish orange (5C6), pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat to umbonate; colony surface powdery to floccose; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (water blue) (24C5); soluble pigment absent; exudate absent; reverse centrally coffee brown (5F7), olive brown (4E5) to yellowish orange (4B7), cream pale yellow (4A3) in margins.

Micromorphology: Conidial heads at first globose, later becoming radiate. Conidiophores uniseriate. Stipes smooth, densely covered by short hairs in SEM, 6–9 µm wide in the middle part, widening gradually toward the vesicle, non-septate. Vesicles spatulate to clavate, 14–19 µm wide. Phialides flask-shaped, densely covered by short hairs in SEM, 8–10 µm long, covering two thirds to three quarters of the vesicle. Conidia borne smooth, ellipsoidal, becoming rough-walled, tuberculate in SEM, subglobose, ovate or barrel-shaped at maturity, connectives evident, 4–5 × 3–4 µm.

Distinguishing characters: See distinguishing characters of *A. penicillioides*. Isolates of *A. clavatophorus* have larger conidia (on average 4.8 × 3.5 µm) compared to *A. tardicrescens* (on average 4 × 2.9 µm) and smaller vesicles (on average 16.5 µm) compared to *A. magnivesiculatus* (on average 21.4 µm). The shape of the vesicles is usually spatulate to clavate in contrast to all other members of the *A. penicillioides* clade that have at least in part pyriform vesicles. Isolates of this species can grow on M60Y at 37 °C.

Additional materials examined: **China**, puerh tea, unknown year of isolation, isolated by J. Houbraken, DTO 257-G5 = CCF 5669 = IBT 34561. **The Netherlands**, unknown substrate, 2014, isolated by M. Meijer, DTO 316-B3. **The Netherlands**, painting, 2014, isolated by J. Houbraken, DTO 324-G2. **USA**, Georgia, Athens, mouldy paper, 1987, isolated by R. T. Hanlin, NRRL 25873 = CCF 5453 = IBT 34632. **USA**, Illinois, Peoria, mouldy cardboard, 1989, collector unknown, NRRL 25875 = CCF 5455.


colony diam (mm)
NaCl concentration (% v/w)
A
1
2
3
B
1
2
C
4
5
6
7
8
9
D
8
9
E
4
5
6
7
F
10
11

Aspergillus conicus Blochwitz in Dale, Ann. Mycol. 12: 38. 1914, emended description. MycoBank MB120214. Fig. 17.

Typus: **Unknown locality of isolation**, unknown substrate, 1924, isolated by P. Biourge [neotype Herb. IMI 172281 designated by Samson & Gams (1985), culture ex-type NRRL 149 = CBS 475.65 = IBT 33667 = DTO 096-H6 = ATCC 16908 = IMI 172281 = CCF 5456].

Colony diam, 14 d (*mm*): M40Y 32–40; CYA (0)10–12; CY20S 5–18; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 18–31; MEA (0)8–10; M60Y 26–40; M60Y 30 °C 26–37; M60Y 37 °C No growth; MEA + 10 % NaCl 16–36.

Colony characters: M40Y 25 °C, 14 d: Colonies flat, wrinkled, surface velutinous; margin filiform; mycelial areas white; sporulation pale turquoise (24A3); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2) to champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: crateriform, wrinkled; colony surface velutinous, margin undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally Persian blue (23A3) to yellowish white (4A2) in margins. CY20S 25 °C, 14 d: cerebriform; colony surface velutinous, margin undulate; mycelial areas white; sporulation light turquoise (24A4); soluble pigment absent; exudate absent; reverse dark turquoise (23F7) to greyish brown (7E3), orange white (5A2) in margins. DG18 25 °C, 14 d: flat, wrinkled; colony surface velutinous to filamentous, margin filiform; mycelial areas white; sporulation bluish grey (23B3); soluble pigment absent; exudate absent; reverse centrally greyish turquoise (24D3) to orange white (5A2) in margins. MEA 25 °C, 14 d: cerebriform; colony surface velutinous, margin undulate; mycelial areas white; sporulation Persian blue (23A3); soluble pigment absent; exudate absent; reverse centrally camel light brown (6D4) to sand greyish yellow (4B3) in margins. M60Y 25 °C, 14 d: flat; colony surface velutinous; margin filiform; mycelial areas white; sporulation pale turquoise (24A3); soluble pigment absent; exudate absent; reverse olive grey (2F2) to lead grey (2D2), champagne greyish yellow (4B4) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat; colony surface velutinous; margin filiform; mycelial areas white; sporulation pale turquoise (24A3); soluble pigment absent; exudate absent; reverse centrally greenish grey (27D2) to sand greyish yellow (4B3) in margins.

Micromorphology: Conidial heads loosely columnar, frequently twisted. Conidiophores uniseriate. Stipes smooth, sparsely covered by short hairs in SEM, 4–5.5 µm wide in the middle part, widening gradually toward the vesicle, with occasional septa. Vesicles pyriform, spatulate to clavate, 8–13 µm wide. Phialides flask-shaped, sparsely covered by short hairs in SEM, 8.5–10 µm long, covering one third to one half of the vesicle. Conidia borne as smooth long cylinders, then becoming delicately rough to definitely aculeate in SEM, globose to subglobose or barrel-shaped, commonly remaining in chains, connectives evident, 4–4.5 × 2.5–3 µm.

Distinguishing characters: *Aspergillus conicus* is most closely related to *A. domesticus* and *A. destruens*. The colour of sporulating colonies on M40Y is pale turquoise in *A. conicus*, while myrtle green in *A. destruens*; reverse of *A. conicus* is olive grey in the centre, but apricot greyish orange in *A. destruens*. These three species share almost identical micromorphology, but differences can be found in their response to the osmotic stress. *Aspergillus conicus* grows fastest on MEA + 5 % NaCl and MEA + 10 % NaCl, while *A. domesticus* grows slowest (see Fig. 12E). *Aspergillus gracilis* and *A. pseudogracilis* differ from these three species by their inability to grow on MEA and CYA at 25 °C (several strains of *A. gracilis* exhibited very restricted growth on CYA) and more rapid growth on M60Y at 25 °C and 30 °C. In contrast to *A. domesticus*, isolates of *A. conicus* can grow on MEA and grow slightly faster on CYA. *Aspergillus villosus* can be distinguished from *A. conicus*, *A. domesticus* and *A. destruens* by its faster growth on CY20S, ability to grow on CY20S at 30 °C and on M60Y at 37 °C.

Additional materials examined: **Belgium**, candy, 2004, collector unknown, IHEM 20709. **Belgium**, Braine-l'Alleud, wooden statue, 2000, collector unknown, IHEM 16531. **Czech Republic**, kernel of *Bertholletia excelsa* (Brazil nut), unknown year of isolation, isolated by L. Marvanová, CCF 4042. **Denmark**, near Copenhagen, air in bathroom, 2009, isolated by J. C. Frisvad, DTO 110-C5. **Denmark**, near Copenhagen, air in living room, 2009, isolated by J. C. Frisvad, DTO 110-F5 = CCF 5667 = IBT 34534. **France**, French madeleines duchesse, 2013, isolated by F. Déniel, CBS 140429 = UBOCC-A-115041 = DTO 334-D9. **Germany**, Koln, air, unknown year of isolation, collector unknown, DTO 077-H2 = CBS 117332. **Germany**, indoor air, 2014, isolated by U. Hack, DTO 321-H2, DTO 321-H5. **India**, Tamil Nadu, Tiruchirapally, *Musa acuminate*, unknown year of isolation, DTO 079-A9 = CBS 122.453. **Israel**, Eliat, microbial mat, 2008, isolated by R. Tkavc, EXF-5015 = CCF 5650 = IBT 34273. **Puerto Rico**, Bayamon, office air, 2014, isolated by Ž. Jurjević, CCF 5461 = EMSL No. 2549. **Slovenia**, Ljubljana, oil painting on canvas, 2012, isolated by P. Zalar, EXF-7663 = IBT 34267 = IBT 33574, EXF-7660 = IBT 34263 = IBT 33577. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar and D.D. Graf, EXF-10387 = CCF 5651 = IBT 34289. **The Netherlands**, Witten, indoor air, unknown year of isolation, isolated by C. Trautmann and I. Dill, DTO 077-H5. **The Netherlands**, Zwartewaal, museum piece, 2012, isolated by M. Meijer, DTO 231-C2, DTO 231-C3 = CCF 5665. **The Netherlands**, Eindhoven, indoor air, 2006, isolated by J. Houbraken, DTO 017-B9. **USA**, Illinois, Chicago, asphalt roof shingle, 1959, isolated by H. G. Hedrick, NRRL 25848. **USA**, Delaware, air, pineapple room, warehouse, 2012, isolated by Ž. Jurjević, CCF 5459 = EMSL No. 1649. **USA**, California, home air, 2011, isolated by Ž. Jurjević, CCF 5458 = EMSL No. 1490. **USA**, Idaho, home air, 2010, isolated by Ž. Jurjević, CCF 5457 = NRRL 62007 = EMSL No. 1318. **USA**, New York, unknown substrate, 1975, isolated by I. Weitzman, NRRL 25881. **USA**, Pennsylvania, West Chester, living room air, 2013, isolated by Ž. Jurjević, CCF 5460 = EMSL No. 2217. **USA**, Georgia, Kennesaw, basement, 2015, isolated by Ž. Jurjević, EMSL No. 3245. **USA**, Missouri, Saint Louis, bedroom, wood base, 2015, isolated by Ž. Jurjević, EMSL No. 3209. **Unknown locality of isolation**, unknown substrate, 2007, isolated by T. van Doorn, DTO 039-G7.

Aspergillus destruens Zalar, F. Sklenar, S.W. Peterson & Hubka, sp. nov. MycoBank MB818930. Fig. 18.

Etymology: Named after the common occurrence of this species on old deteriorated paintings.

Typus: **USA**, Maryland, maize seed, 1917, isolated by C. V. Piper (holotype PRM 944428, isotype PRM 944429, culture ex-type NRRL 145 = IMI 358691 = CCF 5462 = CBS 593.91 = DTO 079-A8 = IBT 34818).

Colony diam, 14 d (*mm*): M40Y 19–36; CYA 5–10; CY20S 6–13; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 14–30; MEA (0) 4–7; M60Y 34–41; M60Y 30 °C 16–41; M60Y 37 °C No growth; MEA + 10 % NaCl 20–30.

Fig. 12. Osmotic gradient growth curves of species from *A. restrictus*, *A. conicus* and *A. vitricola* clades. Growth curves were created using local regression (LOESS) in R 3.3.1, grey zones represent 95 % confidence intervals. 1 – *A. restrictus*; 2 – *A. caesiellus*; 3 – *A. pachycaulis*; 4 – *A. conicus*; 5 – *A. domesticus*; 6 – *A. destruens*; 7 – *A. villosus*; 8 – *A. gracilis*; 9 – *A. pseudogracilis*; 10 – *A. vitricola*; 11 – *A. glabripes*.

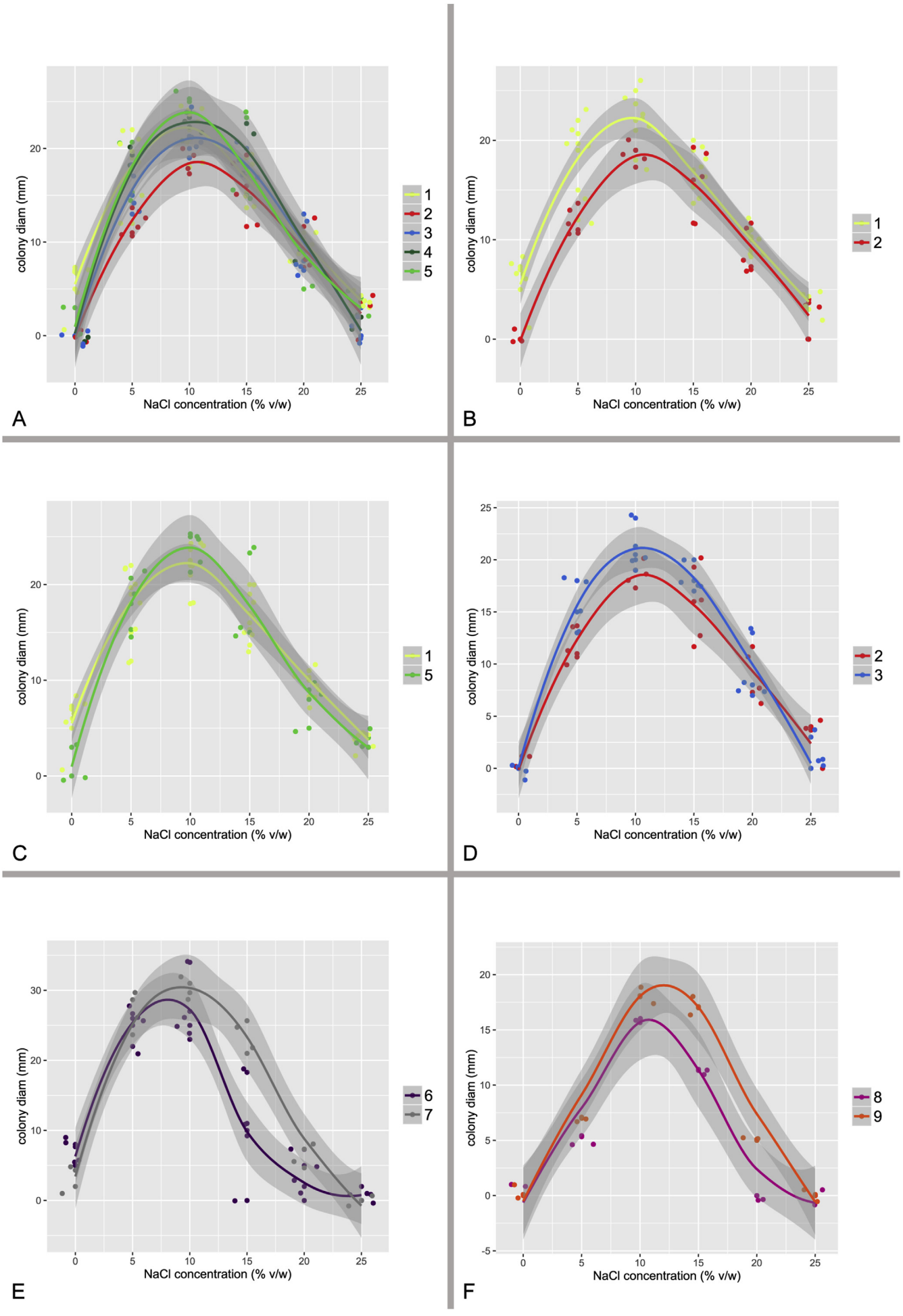

colony diam (mm)
NaCl concentration (% v/w)
1
2
3
4
5
A
B
C
D
E
F
6
7
8
9

Table 7. Overview of colony characters on M40Y medium.

Species	Sporulation colour	Reverse colour in the center	Shape of conidial heads
***A. restrictus* clade**			
A. restrictus	Greyish turquoise (24C4)	Cinnamon brown (6D6)	Columnar
A. caesiellus	Greyish turquoise (24E4)	Sunburn light brown (6D5)	Columnar
A. pachycaulis	Greyish turquoise (24C4)	Brownish yellow (5C8)	Columnar
***A. conicus* clade**			
A. conicus	Pale turquoise (24A3)	Olive grey (2F2)	Loosely columnar
A. destruens	Myrtle green (25F7)	Apricot greyish orange (5B6)	Loosely columnar
A. domesticus	Aquamarine greyish turquoise (24B3)	Olive grey (2F2)	Loosely columnar
A. gracilis	Aquamarine greyish turquoise (24B3) to turquoise white (24A2)	Olive grey (2F2)	Loosely columnar
A. pseudogracilis	Light turquoise (24A4)	Cinnamon brown (6D6)	Loosely columnar
A. villosus	Greyish green (25E7) to bluish green (25A6)	Olive green (2F6)	Loosely columnar
***A. vitricola* clade**			
A. vitricola	Greyish turquoise (water blue) (24C5)	Olive green (2F6)	Radiate
A. glabripes	Yellowish green (29C7)	Dark brown (6F7)	Radiate
***A. penicillioides* clade**			
A. penicillioides	Deep green (25E8)	Coffee brown (5F7)	First globose, later becoming loosely columnar
A. canadensis	Jungle green (25F5) to pale turquoise (24A3)	Olive grey (2F2)	Radiate
A. clavatophorus	Greyish green (28D6)	Coffee brown (5F7)	First globose, later becoming radiate
A. hordei	Dark turquoise (24F8)	Cuba reddish brown (9E8)	First globose, later becoming loosely columnar
A. infrequens	Pale turquoise (24A3) to dark turquoise (24F8)	Dark green (30F8)	Radiate
A. magnivesiculatus	Greyish green (26D5)	Mahogany red (8E7)	First globose, later becoming loosely columnar to radiate
A. reticulatus	Greyish turquoise (24E6)	Golden brown (5D7)	Globose to radiate
A. salinicola	Greyish green (25B5)	Golden brown (5D7)	First globose, later becoming loosely radiate
A. tardicrescens	Greyish turquoise (water blue) (24C5)	Olive brown (4F8)	First globose, later becoming radiate

Colony characters: M40Y 25 °C, 14 d: Colonies centrally raised and winkled, surface velutinous; margin irregular and delicately filiform; mycelial areas white; sporulation myrtle green (25F7); soluble pigment absent; exudate absent; reverse centrally apricot greyish orange (5B6) to light yellow (4A4) in margins. CYA 25 °C, 14 d: cerebriform; colony surface velutinous, margin undulate; mycelial areas white; sporulation turquoise white (24A2); soluble pigment absent; exudate absent; reverse centrally sky grey (23B2) to cream pale yellow (4A3) in margins. CY20S 25 °C, 14 d: cerebriform to crateriform; colony surface velutinous, margin entire to delicately filiform; mycelial areas white; sporulation light turquoise (24A4); soluble pigment absent; exudate absent; reverse greyish turquoise (24E7) to pale orange (5A3) in margins. DG18 25 °C, 14 d: craterifiorm; colony surface floccose, margin entire to undulate; mycelial areas white; sporulation aquamarine greyish turquoise (23B3); soluble pigment greyish yellow (2B3); exudate absent; reverse centrally dull green (27E3) to yellowish white (4A2) in margins. MEA 25 °C, 14 d: cerebriform; colony surface velutinous, margin entire; sporulation greenish grey (25B2); soluble pigment absent; exudate absent; reverse centrally camel olive grey (2F2) to sand dark yellow (4C8) in margins. M60Y 25 °C, 14 d: umbonate; colony surface floccose to filamentous; margin entire to delicately filiform; mycelial areas white; sporulation pastel green (25B4); soluble pigment absent; exudate absent; reverse cinnamon brown (6D6) to champagne greyish yellow (4B4). MEA + 10 % NaCl 25 °C, 14 d: raised, irregularly wrinkled; colony surface velutinous; margin delicately filiform; mycelial areas white; sporulation bluish grey (23B3); soluble pigment absent; exudate absent; reverse centrally greenish grey (27D2) to cream pale yellow (4A3) in margins.

Micromorphology: Conidial heads loosely columnar. Conidiophores uniseriate. Stipes smooth, densely covered by long hairs in SEM, 4–6 µm wide in the middle part, widening gradually toward the vesicle, with occasional septa. Vesicles predominantly ellipsoidal to pyriform, less commonly spatulate to clavate,

Fig. 13. Osmotic gradient growth curves of species from *A. penicillioides* clade. Growth curves were created using local regression (LOESS) in R 3.3.1, grey zones represent 95 % confidence intervals. 1 – *A. penicillioides*; 2 – *A. tardicrescens*; 3 – *A. clavatophorus*; 4 – *A. magnivesiculatus*; 5 – *A. hordei*; 6 – *A. reticulatus*; 7 – *A. salinicola*; 8 – *A. canadensis*; 9 – *A. infrequens*.

Table 8. Growth rate comparison after 14 d (mm).

Species	CYA, 25 °C	MEA, 25 °C	MEA+10 % NaCl, 25 °C	DG18, 25 °C	M40Y, 25 °C	CY20S			M60Y		
						25 °C	30 °C	37 °C	25 °C	30 °C	37 °C
***A. restrictus* clade**											
A. restrictus	12–16	5–13	33–40	28–33	42–53	24–29	12–25	0	38–50	45–55	20–40
A. caesiellus	11–22	17–22	36–45	32–38	58–60	21–35	12–40	0	48–60	45–60	32–55
A. pachycaulis	11–26	21–31	27–43	35–48	20–60	15–40	22–36	(0) 8–10	25–60	36–60	38–60
***A. conicus* clade**											
A. conicus	(0) 10–12	(0) 8–10	16–36	18–31	32–40	5–18	0	0	26–40	26–37	0
A. destruens	5–10	(0) 4–7	20–30	14–30	19–36	6–13	0	0	34–41	16–41	0
A. domesticus	0–8	0	23–31	20–24	36–38	5–17	0	0	29–38	15–30	0
A. gracilis	0–8	0	12–24	9–16	30–42	7–13	0	0	38–44	39–45	0
A. pseudogracilis	0	0	25–26	20–22	42–45	7–8	0	0	52–53	43–45	0
A. villosus	8–10	5–6	22–23	16–20	21–23	17–19	7–8	0	26–28	28–30	6–8
***A. vitricola* clade**											
A. vitricola	0	0	9–26	8–18	19–36	(0) 4–7	0	0	25–42	16–42	0
A. glabripes	3–7	4–11	22–28	15–25	38–52	8–18	5–16	0	35–40	41–56	(0) 7–10
***A. penicillioides* clade**											
A. penicillioides	7–9	5–9	24–37	17–36	27–43	11–20	11–15	0	28–59	40–60	17–45
A. canadensis	0	0	17–33	7–15	25–29	(0) 10–11	0	0	23–42	2–20	0
A. clavatophorus	0	0	18–28	18–25	27–33	7–16	0	0	35–44	29–54	(0) 5–17
A. hordei	0–8	0–5	26–29	13–26	25–29	(0) 13–16	(0)8–15	0	31–47	25–48	22–33
A. infrequens	4–5	0	18–19	25–26	21–22	7–8	0	0	40–43	8–10	0
A. magnivesiculatus	0	0	27–34	20–26	27–35	11–17	0	0	47–60	40–50	0
A. reticulatus	8–14	2–7	32–40	26–33	46–50	21–30	8–18	0	55–60	50–55	3–20
A. salinicola	(0) 8–9	0–5	32–35	21–30	32–40	9–20	0–5	0	42–55	27–43	0
A. tardicrescens	0	0	19–29	12–17	17–29	0–5	0	0	31–50	15–35	0

8–14 µm wide. Phialides flask-shaped, densely covered by long hairs in SEM, 8.5–11.5 µm long, covering one to two thirds of the vesicle. Conidia borne as smooth, long cylinders, becoming delicately rough and definitely echinulate at maturity, aculeate in SEM, subglobose to ovate, commonly remaining in chains, connectives evident, 3.5–5.5 × 2.5–3.5 µm.

Distinguishing characters: See distinguishing characters of *A. conicus*. *Aspergillus destruens* is most closely related to *A. conicus* and *A. domesticus*, but even though the latter two are morphologically similar, they are different from *A. destruens*. The colour of sporulating colonies of *A. destruens* on M40Y and M60Y is darker, the reverse colour is apricot greyish orange in the centre in contrast to olive grey in the other two species.

Additional materials examined: **Hungary**, indoor air, 2009, collector unknown, DTO 147-E2. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar and D.D. Graf, EXF-10350 = IBT 34270, EXF-10407 = CCF 5652 = IBT 34285, EXF-10411 = IBT 34265, EXF-10390 = IBT 34276, EXF-10017 = IBT 33576. **Slovenia**, Ljubljana, oil painting on canvas, 2012, isolated by P. Zalar and D.D. Graf, EXF-7699 = IBT 34262, EXF-7651 = CCF 5653 = IBT 34258, EXF-7661 = IBT 34271 = IBT 33573, EXF-7667 = IBT 34288, EXF-7703 = IBT 34259, EXF-7657 = IBT 34264, EXF-10431 = IBT 34257, EXF-7666 = IBT 34268. **The Netherlands**, Utrecht, air in villa, unknown year of isolation, collector unknown, DTO 254-B2 = IBT 34522. **The Netherlands**, Tilburg, air in bakery, 2012, collector unknown, DTO 220-B2. **The Netherlands**, surface of cheese, 2011, isolated by J. Houbraken, DTO 161-B7 = CCF 5671. **The Netherlands**, Tilburg, air in bakery, 2009, isolated by J. Houbraken, DTO 113-E7 = CCF 5668. **The Netherlands**, Leerdam, unknown substrate, unknown year of isolation, isolated by M. Meijer, DTO 303-H2. **USA**, unknown substrate, 1939, isolated by C.W. Emmons, NRRL 157 = CCF 5463.

Aspergillus domesticus F. Sklenar, Houbraken, Zalar & Hubka, sp. nov. MycoBank MB818931. Fig. 19.

Etymology: Referring to the common occurrence of this species in indoor environments.

Typus: **The Netherlands**, Tiel, wallpaper, 2008, collector unknown (holotype PRM 944426, isotype PRM 944427, culture ex-type DTO 079-F2 = CCF 5464 = NRRL 66616 = IBT 34814).

Colony diam, 14 d (*mm*): M40Y 36–38; CYA 0–8; CY20S 5–17; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 20–24; MEA No growth; M60Y 29–38; M60Y 30 °C 15–30; M60Y 37 °C No growth; MEA + 10 % NaCl 23–31.

Colony characters: M40Y 25 °C, 14 d: Colonies flat, wrinkled in the centre, surface velutinous; margin filiform; mycelial areas white; sporulation aquamarine greyish turquoise (24B3); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2) to champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: crateriform; colony surface velutinous, margin undulate; mycelial areas white; sporulation turquoise white (24A2); soluble pigment absent; exudate absent; reverse centrally fog blue (23C3) to orange white (5A2) in margins. CY20S 25 °C, 14 d: cerebriform; colony surface velutinous, margin entire to delicately filiform; mycelial areas white; sporulation light turquoise (24A5) to turquoise white (24A2); soluble pigment absent; exudate absent; reverse dark turquoise (23F7) to fog blue (23C3), pale orange (5A3) in margins. DG18 25 °C, 14 d:

Table 9. Exometabolites found in members of *Aspergillus* section *Restricti*.

Species	Strain number[1,2]	Exometabolites[3]
A. caesiellus	NRRL 5061^T = CBS 470.65 = DTO 093-H3 = ATCC 11905 = IMI 172278 = CCF 5447 = IBT 34620	Asperphenamate, indole alkaloid "A" & "B", CADA1, CADA2
	DTO 025-I4 = IBT 34538	Asperphenamate, CAS1-CAS5
	CCF 5448 = EMSL No. 1383 = IBT 34621	Asperphenamate, indole alkaloid "A", CA1, CA2, CAD1, CADA2, CAS1-CAS4
	CCF 5451 = EMSL No. 1650 = IBT 34622	Asperphenamate, indole alkaloid "A" & "B", CA1, CAS1-CAS5
A. canadensis	CCF 5548^T = KAS 6194 = DTO 356-H9 = IBT 34520 = IBT 34642 = NRRL 66614	Asperglaucide, aurantiamide[4], aurantiamide "B", chrysogine, indole alkaloid "A", CAN1
	KAS 7710 = DTO 357-B4 = CCF 5552 = IBT 34636	Asperglaucide, aurantiamide, indole alkaloid "A"
	KAS 7708 = DTO 357-B2 = CCF 5550 = IBT 34637	asperglaucide, indole alkaloid "A", ASPHAM1
	KAS 7719 = DTO 357-C2 = CCF 5553 = IBT 34638	Asperglaucide, aurantiamide , indole alkaloid "A", ASPHAM1, CANA1-CANA4
	KAS 7706 = DTO 357-A9 = CCF 5549 = IBT 34639 = NRRL 66615	Asperglaucide, aurantiamide , indole alkaloid "A", ASPHAM1
	KAS 7709 = DTO 357-B3 = CCF 5551 = IBT 34640	Asperglaucide, asperphenamate, indole alkaloid "A"
	KAS 7720 = DTO 357-C3 = IBT 34641	Asperglaucide, aurantiamide
A. clavatophorus	NRRL 25874^T = CCF 5454 = IBT 34560 = DTO 356-D8 = IBT 34823	Asperglaucide, aurantiamide, indole alkaloid "A"
	DTO 257-G5 = CCF 5669 = IBT 34561	Asperglaucide, PRO1, Raistrick phenol "A"
	NRRL 25873 = CCF 5453 = IBT 34632	Asperglaucide, aurantiamide, PRO1, Raistrick phenol "B"
A. conicus	NRRL 149^T = CBS 475.65 = IBT 33667 = DTO 096-H6 = ATCC 16908 = IMI 172281 = CCF 5456	Asperglaucide, asperphenamate, indole alkaloid "A", phthalide
	DTO 110-F5 = CCF 5667 = IBT 34534	Asperphenamate, phthalide, PRO2
	EXF-7660 = IBT 34263 = IBT 33577	Asperphenamate, phthalide, WOVI, PITAL
	EXF-7663 = IBT 34267 = IBT 33574	Asperphenamate, phthalide, clavatol "A", FOLS, indole alkaloid "B", PITAL
	EXF-5015 = CCF 5650 = IBT 34273	Asperphenamate
	EXF-10387 = CCF 5651 = IBT 34289	Asperglaucide, PI-4 (fulvic acid biosynthetic family)
A. destruens	NRRL 145^T = IMI 358691 = CCF 5462 = CBS 593.91 = DTO 079-A8 = IBT 34818	Asperglaucide, chromanol "X", naphtho-gamma-pyrone "A", YELLO
	DTO 254-B2 = IBT 34522	Asperphenamate, clavatol "B" & "C", pyrone "A", "B", "C", "D", "E", "F", "G", "H" & "I", WOVI
	EXF-7657 = IBT 34264	Asperphenamate, pyrone "A", pyrone "G", WOVI
	EXF-7703 = IBT 34259	Asperphenamate, clavatol "D"
	EXF-7651 = CCF 5653 = IBT 34258	Asperphenamate, pyrone "B", "D" & "G", clavatol "A"
	EXF-7699 = IBT 34262	Asperphenamate, pyrone "A", pyrone "B" & "G", clavatol "A"
	EXF-7666 = IBT 34268	Asperphenamate
	EXF-7661 = IBT 34271 = IBT 33573	Asperphenamate, clavatol "A", ASPHAM1, CAS1, CAS2, naphtho-gamma-pyrone "A"
	EXF-7667 = IBT 34288	Asperphenamate, pyrone "G"
	EXF-10431 = IBT 34257	Asperphenamate, pyrone "A", "B" & "G"
	EXF-10411 = IBT 34265	Asperphenamate, pyrone "A", "B", "E" & "G"
	EXF-10350 = IBT 34270	Asperphenamate, WOVI
	EXF-10407 = CCF 5652 = IBT 34285	Asperglaucide
	EXF-10017 = IBT 33576	Asperphenamate, ASPHAM1, naphtho-gamma-pyrone "A", WOVI
	EXF-10390 = IBT 34276	Asperphenamate, clavatol "A", SKIZ1, SKIZ2, CAS5
A. domesticus	DTO 079-F2^T = CCF 5464 = NRRL 66616 = IBT 34814	Asperphenamate, clavatol "D", FOLS
	CBS 233.90 = DTO 079-A7 = IBT 34515	Asperphenamate, WOVI
	CBS 116419 = DTO 077-G6 = IBT 34535	Asperphenamate, indole alkaloid "B", FOLS
	EXF-10012 = IBT 34274	Asperphenamate, clavatol "D", FOLS, indole alkaloid "B"
	EXF-10583 = IBT 34275	Asperphenamate, indole alkaloid "B"
A. glabripes	CCF 5474^T = DTO 356-E8 = EMSL No. 2462 = NRRL 66618 = IBT 34820	SCUB, STALD1, STALD2, FJOS, indole alkaloid "A", PRO1
	CCF 5469 = EMSL No. 1483 = DTO 356-E5 = IBT 34519 = IBT 34821 = NRRL 66619	Mycophenolic acid, FAESI, GUOY, GUOY2, PRO1, SCUB, SPOJN1, SPOJN2, STALD1-STALD3, TERRIT1-TERRIT3, XANS
	UBOCC-A-116021 = CCF 5467 = IBT 34625	Indole alkaloid "A", PITAL
	CCF 5473 = EMSL No. 2442 = IBT 34626	Asperphenamate, indole alkaloid "A", PRO1, SCUB
	IBT 26603[5]	SCUB, STALD1, STALD2, FJOS, indole alkaloid "A", PRO1
A. gracilis	NRRL 4962^T = CBS 539.65 = DTO 351-H7 = CCF 5478 = ATCC 16906 = IMI 211393 = IBT 34817	Asperphenamate, CHE1-CHE3, chromanol "X", clavatol "D", indole alkaloid "B", FOL1, FOL2, FOLS, SCAB
	CCF 5479 = EMSL No. 2775 = DTO 356-F4 = IBT 34559	Asperphenamate, mycophenolic acid, FOL2, FOLS, indole alkaloid "B", SCAB
	CCF 5482 = EMSL No. 2923 = IBT 34623	Indole alkaloid "A", SCAB
A. halophilicus	NRRL 2739^T = CCF 5687 = ATCC 16401 = CBS 12262 = IMI 211802 = IBT 34878	Asperphenamate, echinulin, preechinulin, cristatin A, pyrone "R", "U", "V", "W", "X", "Z", cristatin "X", DYLD1-DYLD4, PITAL
	DTO 271-F4 = CCF 5825 = IBT 34881	Asperphenamate, echinulin, preechinulin, cristatin A, trace of mycophenolic acid, pyrone "K", "L", "M", "N", "O", "P", "R", "S" & "T", indole alkaloid "A", cristatine "X", PITAL
	MUT 562	

(*continued on next page*)

Table 9. (Continued).

Species	Strain number[1,2]	Exometabolites[3]
		Asperphenamate, cristatin A, echinulin, pyrone "K", "N", "U", "V", "W", "X", "R", DYLD1-DYLD4, PITAL
	MUT 1305	Asperphenamate, cristatin A, echinulin, pyrone "K", "N", "R", "V", "W", "X", DYLD1-DYLD4, PITAL
	MUT 798	Asperphenamate, cristatin A, echinulin, pyrone "K", "M", "N", "O", "P", "R", DYLD1-DYLD4, PITAL
	MUT 1307	Asperphenamate, cristatin A, echinulin, pyrone "K", "N", "R", "U", "V", "W", "X", "Y", "Z", DYLD1-DYLD4, PITAL
A. hordei	NRRL 25825^T = CCF 5483 = DTO 356-D3 = IBT 34539	Asperglaucide, aurantiamide, aurantiamide "B", cristatine A, echinulin, indole alkaloid "C", "D" & "E"
	DTO 281-A4 = IBT 34517	Asperglaucide, aurantiamide, chromanol "X", cristatin A, echinulin, YEY
	NRRL 25830 = CCF 5485 = IBT 34631	Asperglaucide, aurantiamide, aurantiamide "B", echinulin, cristatin A, KOS3, PENIT
A. infrequens	NRRL 25868^T = CCF 5486 = DTO 356-D6 = IBT 34524	Asperphenamate
A. magnivesiculatus	NRRL 25866^T = CCF 5488 = IBT 34816	Asperglaucide, cristatin A, echinulin, indole alkaloid "A", KOS1-KOS3
	CCF 5489 = EMSL No. 1315 = DTO 356-E2 = IBT 34516	Antarone A, asperglaucide, cristatin A, echinulin, FU1, FU2, KOS1-KOS3, FUSM1, FUSM2
	EXF-10353 = IBT 34284	Asperglaucide, pyrone "A", "B", "D" & "G"
	EXF-10356 = IBT 34279	Asperglaucide, pyrone "G" & "I"
	EXF-10347 = IBT 34283	Asperglaucide
A. pachycaulis	NRRL 25824^T = CCF 5492 = DTO 356-D2 = IBT 34521 = IBT 34812	Asperphenamate, indole alkaloid "A" & "B", phthalide, mycophenolic acid, ASPHAM2, CACCA, OSM1, OSM2, clavatol "D", FIMUI, naphtho-gamma-pyrone "C" & "D"
	CCF 5493 = EMSL No. 2310 = DTO 356-E6 = IBT 34536	Asperphenamate, phthalide, indole alkaloid "A" and "B"
A. penicillioides	NRRL 4548^T = CBS 540.65 = ATCC 16910 = IMI 211342 = DTO 207-I7 = CCF 5494 = IBT 34627	Asperglaucide, aurantiamide
	DTO 267-A4 = IBT 34614	Asperglaucide, cristatine A, trace of mycophenolic acid, YEY, echinulin
	CCF 5498 = EMSL No. 2440 = DTO 356-E7 = IBT 34815	Asperglaucide
	CCF 5497 = EMSL No. 2430 = IBT 34628	Asperglaucide, aurantiamide, cristatine A, YEY
	CCF 5501 = EMSL No. 2749 = IBT 34629	Asperglaucide, YEY, echinulin, met k
	CCF 5500 = EMSL No. 2651 = IBT 34630	Asperglaucide, YEY
A. pseudogracilis	CCF 5505^T = EMSL No. 2765 = DTO 356-F3 = NRRL 66620 = IBT 34813	Asperphenamate, indole alkaloid "B", FOLS
A. restrictus	NRRL 154^T = CBS 117.33 = CBS 541.65 = DTO 079-B2 = ATCC 16912 = IHEM 3920 = IMI 16267 = CCF 5506 = IBT 34615	Asperphenamate, CACCA, indole alkaloid "A" & "B"
	DTO 065-C7 = IBT 34541	Asperphenamate, indole alkaloid "B", CRYPT1, CRYPT2, FNOVL1, FNOVL2
	CCF 5511 = EMSL No. 1675 = IBT 34616	Asperphenamate, FNOVL2, FOLS, indole alkaloid "B"
	CCF 3364 = IBT 34619	Asperphenamate, CRYPT1, CRYPT2, CRYPT4, FNOVL1, FNOVL2, FOLS, indole alkaloid "B"
	CCF 5507 = EMSL No. 1379 = IBT 34618	Asperphenamate, indole alkaloid "B", FOLS
	CCF 5512 = EMSL No. 2206 = IBT 34617	Asperphenamate, CRYPT1-CRYPT5, FNOVL1-FNOVL3, PALO1-PALO3
A. reticulatus	NRRL 25852^T = CCF 5516 = DTO 356-D4 = IBT 34540	Asperglaucide, aurantiamide, indole alkaloid "A", clavatol "D"
	CCF 5521 = EMSL No. 1362 = DTO 356-E4 = IBT 34518	Asperglaucide, aurantiamide, indole alkaloid "A", PITAL
	CCF 5524 = EMSL No. 2548 = IBT 34637	Asperglaucide, aurantiamide, clavatol "D"
	CCF 3112 = IBT 34634 = NRRL 62490	Asperglaucide, aurantiamide, indole alkaloid "A", cyclopaldic acid chromanols
	CCF 5525 = NRRL 58572 = EMSL No. 885 = IBT 34880	Asperglaucide, cyclopaldic acid, cyclopaldic acid chromanols
	CCF 5518 = NRRL 58903 = EMSL No. 1272 = IBT 34819	Asperglaucide, aurantiamide, indole alkaloid "A", cyclopaldic acid chromanols
A. salinicola	EXF-10401^T = IBT 34266 = NRRL 66621 = CCF 5526	Antarone A, asperentin "A", "B", "C", "D", "E", "F", "G", "H" & "I", asperglaucide, aurantiamide, met k, SOSO
	EXF-226 = CCF 5527 = IBT 34277 = NRRL 66622	Asperglaucide, aurantiamide, indole alkaloid "A"
	UBOCC-A-116019 = CCF 5528 = IBT 34635	Asperglaucide, aurantiamide
A. tardicrescens	DTO 316-B5^T = CCF 5529 = NRRL 66623 = IBT 34558	Asperglaucide, mycophenolic acid, clavatol "D", FOL2, indole alkaloid "A"
	DTO 316-A8 = IBT 34562	Asperglaucide, mycophenolic acid, FOL2, KOS3, indole alkaloid "A", indole alkaloid "F"

Table 9. (Continued).

Species	Strain number[1,2]	Exometabolites[3]
	EXF-10454 = IBT 34281 = CCF 5530 = NRRL 66624	Asperfuran, asperglaucide, echinulin, indole alkaloid "D", FOL2, KOS3
	EXF-10456 = IBT 34286	Asperglaucide
A. villosus	NRRL 25813^T = CCF 5531 = DTO 356-C9 = IBT 34822	Asperphenamate, orthosporin "A", "A1", "D", "F", "G", "H", "I", "J", "K", "L", "M", "N", "O", "P", "Q", "R" & "S", MOL
	UBOCC-A-116020 = CCF 5532 = IBT 34624	Indol alkaloid "A"
	IBT 22529, IBT 22530, IBT 22546[6]	Asperphenamate, orthosporin "A", "F", "G", "O", "R" & "S"
A. vitricola	NRRL 5125^T = DTO 356-F7 = ATCC 16905 = ATCC 36505 = IMI 108298 = CCF 5533 = IBT 34530	No exometabolites detected
	KAS 6150 = DTO 356-H5 = IBT 34531	Orthosporin "A", "B", "C", "D" & "E"
	KAS 6087 = DTO 356-H2 = IBT 34532	Indole alkaloid "G"
	EXF-7700 = CCF 5657 = IBT 34282 = IBT 33575	Asperglaucide, YELL
	EXF-10383 = CCF 5655 = IBT 34272	Asperglaucide, Raistrick phenol "A"
	EXF-10301 = CCF 5654 = IBT 34290	DOKU1, DOKU2

[1] Acronyms of culture collections: ATCC, American Type Culture Collection, Manassas, Virginia, USA; CBS, culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CCF, Culture Collection of Fungi, Charles University, Czech Republic; DTO, working collection of the department of Applied and Industrial Mycology housed at CBS; EMSL, EMSL Analytical Inc., New Jersey, USA; EXF, Culture Collection of Extremophilic Fungi, University of Ljubljana, Slovenia; IBT, Fungal Culture Collection at DTU-Bioengineering, Lyngby, Denmark; BCCM/IHEM, Biomedical Fungi and Yeasts Collection, Scientific Institute of Public Health, Brussels, Belgium; IMI/CABI, International Mycological Institute, Kew, England; KAS, fungal collection of Keith A. Seifert, Ottawa, Canada; MUT, Mycotheca dell'Università degli Studi di Torino, Turin, Italy; NRRL, Agricultural Research Service Culture Collection, Peoria, Illinois, USA; UBOCC, Université de Bretagne Occidentale Culture Collection, Brest, France.
[2] Ex-type strains are designated with superscript T.
[3] Absorption maxima and bracketed retention indexes of all detected exometabolites are listed in Table S1.
[4] Aurantiamide = asperglucide = aurantiamide acetate = lyciumamide = suropeptate.
[5] This isolate originated from mouldy rye bread, Denmark; it was identified solely based on its exometabolite spectrum.
[6] All three isolates originated from whale, Greenland; they were identified solely based on their exometabolite spectra.

umbonate, delicately wrinkled; colony surface powdery to velutinous, margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24C3); soluble pigment absent; exudate absent; reverse centrally peacock blue (24D7) to orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: flat; colony surface velutinous to powdery; margin filiform; mycelial areas white; sporulation turquoise grey (24B2); soluble pigment absent; exudate absent; reverse light blond greyish yellow (4C3). MEA + 10 % NaCl 25 °C, 14 d: flat, wrinkled; colony surface velutinous; margin entire to delicately filiform; mycelial areas white; sporulation light turquoise (24A4); soluble pigment absent; exudate absent; reverse centrally nougat greyish brown (5D3) to sand greyish yellow (4B3) in margins.

Micromorphology: Conidial heads loosely columnar, commonly twisted. Conidiophores uniseriate. Stipes smooth, densely covered by long hairs in SEM, 4–6 µm wide in the middle part, widening gradually toward the vesicle, with occasional septa. Vesicles pyriform, spatulate to clavate, 7–13 µm wide. Phialides flask-shaped, densely covered by long hairs in SEM, 8.5–11 µm long, covering the upper half of the vesicle. Conidia borne as smooth, long cylinders, becoming echinulate at maturity, aculeate in SEM, ellipsoidal or barrel-shaped, commonly remaining in chains, connectives evident, 3.5–4.5 × 2.5–3.5 µm.

Distinguishing characters: See distinguishing characters of *A. conicus*. *Aspergillus domesticus* is most closely related to *A. conicus* and *A. destruens* (Fig. 5). Unlike the two other species, *A. domesticus* does not grow on MEA and also grows slower on CYA.

Additional materials examined: **Belgium**, Brussels, dust from mattress, 1991, collector unknown, IHEM 6549. **France**, French almond madeleines, 2014, isolated by F. Déniel, UBOCC-A-115040 = CBS 140428 = DTO 334-D8, UBOCC-A-115039 = CBS 140427 = DTO 334-D7. **France**, air from the bakery oven room, 2014, isolated by F. Déniel, UBOCC-A-115038. **France**, French mini cakes, 2013, isolated by F. Déniel, UBOCC-A-115048 = CBS 140436 = DTO 334-E7. **France**, Brest, painting from Brittany (painter: E.J. Damery; painting title: Portrait de femme; painting technique: oil on canvas; and painter: J. Gamelin; painting title: Sainte en extase; painting technique: oil on canvas), 2015, isolated by F. Déniel, UBOCC-A-116022 = CCF 5465. **Korea**, *Phragmidium* sp. (rust) on *Rubus coreanus*, 2004, collected by H.D. Shin, isolated by P. Crous, CBS 116419 = DTO 077-G6 = IBT 34535. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar, EXF-10583 = IBT 34275. **Slovenia**, Ljubljana, statue made of wood, textile and sea shells, 2015, isolated by P. Zalar, EXF-10012 = IBT 34274. **The Netherlands**, Zwartewaal, museum piece, 2012, isolated by M. Meijer, DTO 231-C1 = CCF 5666 = NRRL 66617, DTO 231-B9. **The Netherlands**, Gorinchem, archive material, 2008, isolated by J. Houbraken and M. Meijer, DTO 086-D1 = CCF 5670. **The Netherlands**, Paleis het Loo, wallpaper, unknown year of isolation, isolated by J. Varga, CBS 233.90 = DTO 079-A7 = IBT 34515. **USA**, Idaho, home air, 2010, isolated by Ž. Jurjević, CCF 5466 = EMSL No. 1316. **USA**, New Jersey, Freehold, breakfast room, settle plates, 2015, isolated by Ž. Jurjević, EMSL No. 2780.

Aspergillus glabripes F. Sklenar, Ž. Jurjević & Hubka, sp. nov. MycoBank MB818934. Fig. 20.

Etymology: Referring to the absence of fine hairs on the stipe which are visible in majority of sect. *Restricti* species by SEM.

Typus: **Trinidad & Tobago**, Macoya, office folder, 2014, isolated by Ž. Jurjević (holotype PRM 944436, isotype PRM 944437, culture ex-type CCF 5474 = DTO 356-E8 = EMSL No. 2462 = NRRL 66618 = IBT 34820).

Colony diam, 14 d (*mm*): M40Y 38–52; CYA 3–7; CY20S 8–18; CY20S 30 °C 5–16; CY20S 37 °C No growth; DG18 15–25; MEA 4–11; M60Y 35–40; M60Y 30 °C 41–56; M60Y 37 °C (0) 7–10; MEA + 10 % NaCl 22–28.

Colony characters: M40Y 25 °C, 14 d: Colonies raised, surface floccose; margin entire to delicately filiform; mycelial areas white; sporulation yellowish green (29C7); soluble pigment absent; exudate absent; reverse centrally dark brown (6F7) to champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: cerebriform; colony surface floccose, margin undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse pale orange (5A3). CY20S 25 °C, 14 d:

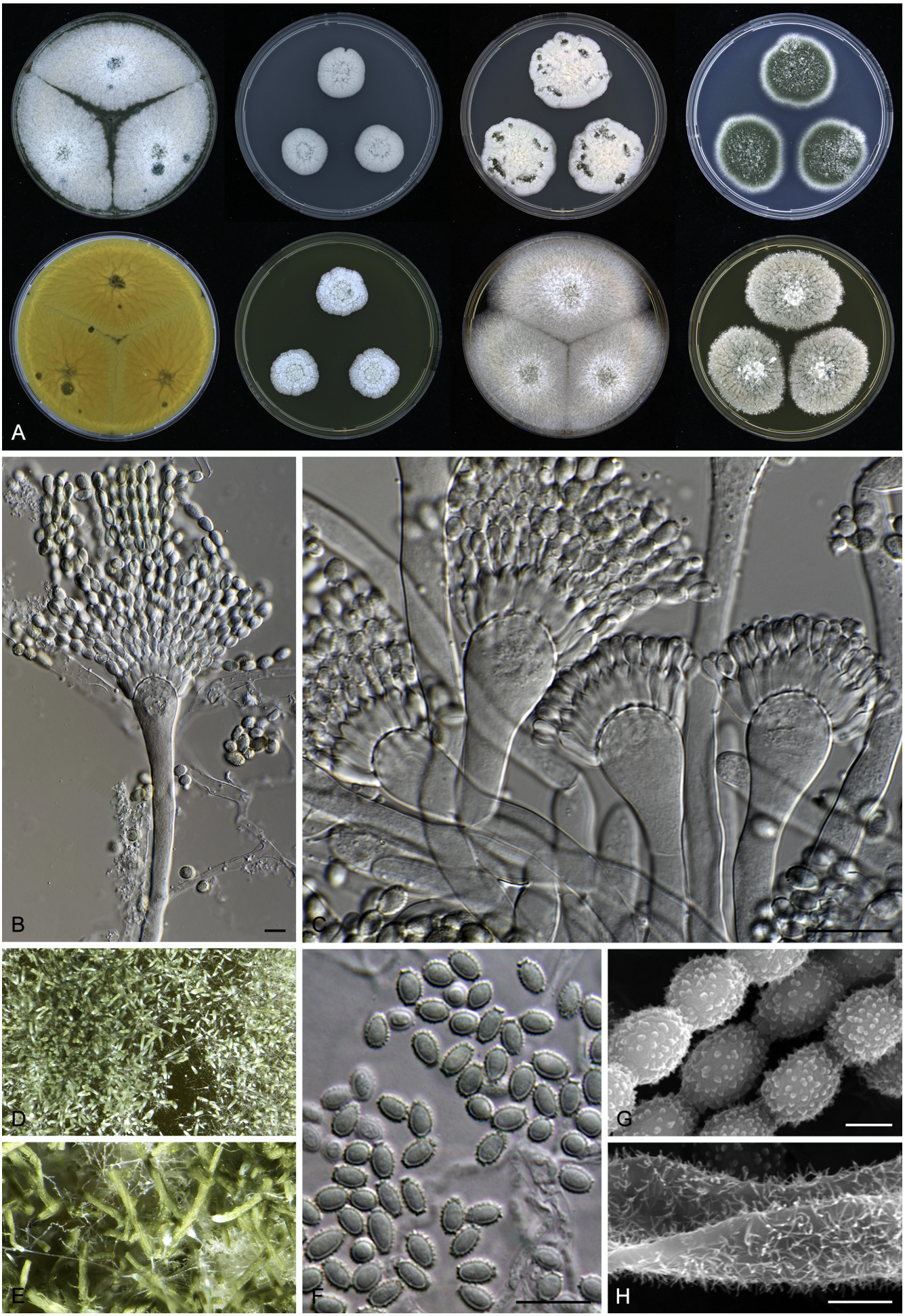


Fig. 14. *Aspergillus caesiellus*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G**. Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

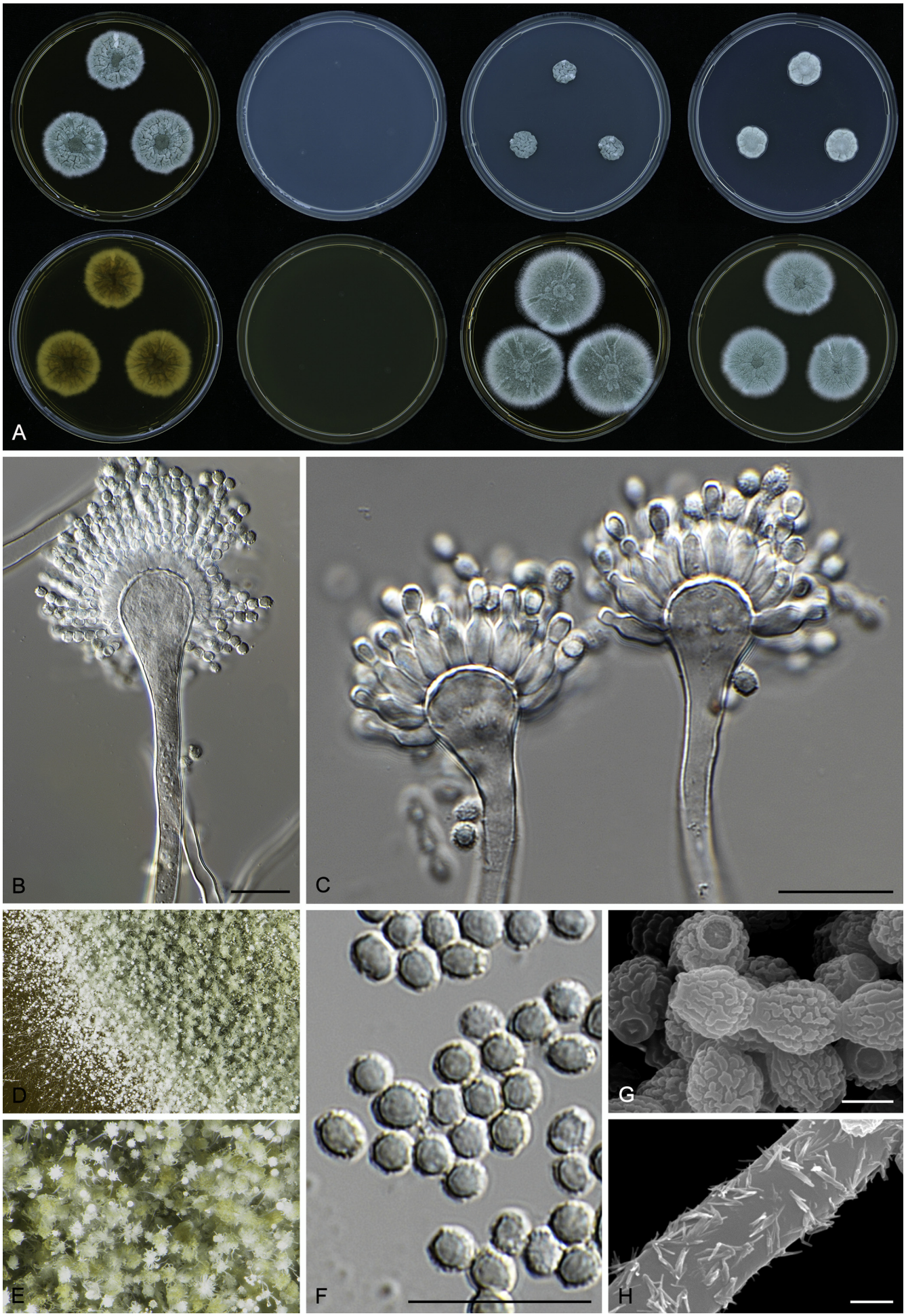


Fig. 15. *Aspergillus canadensis*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

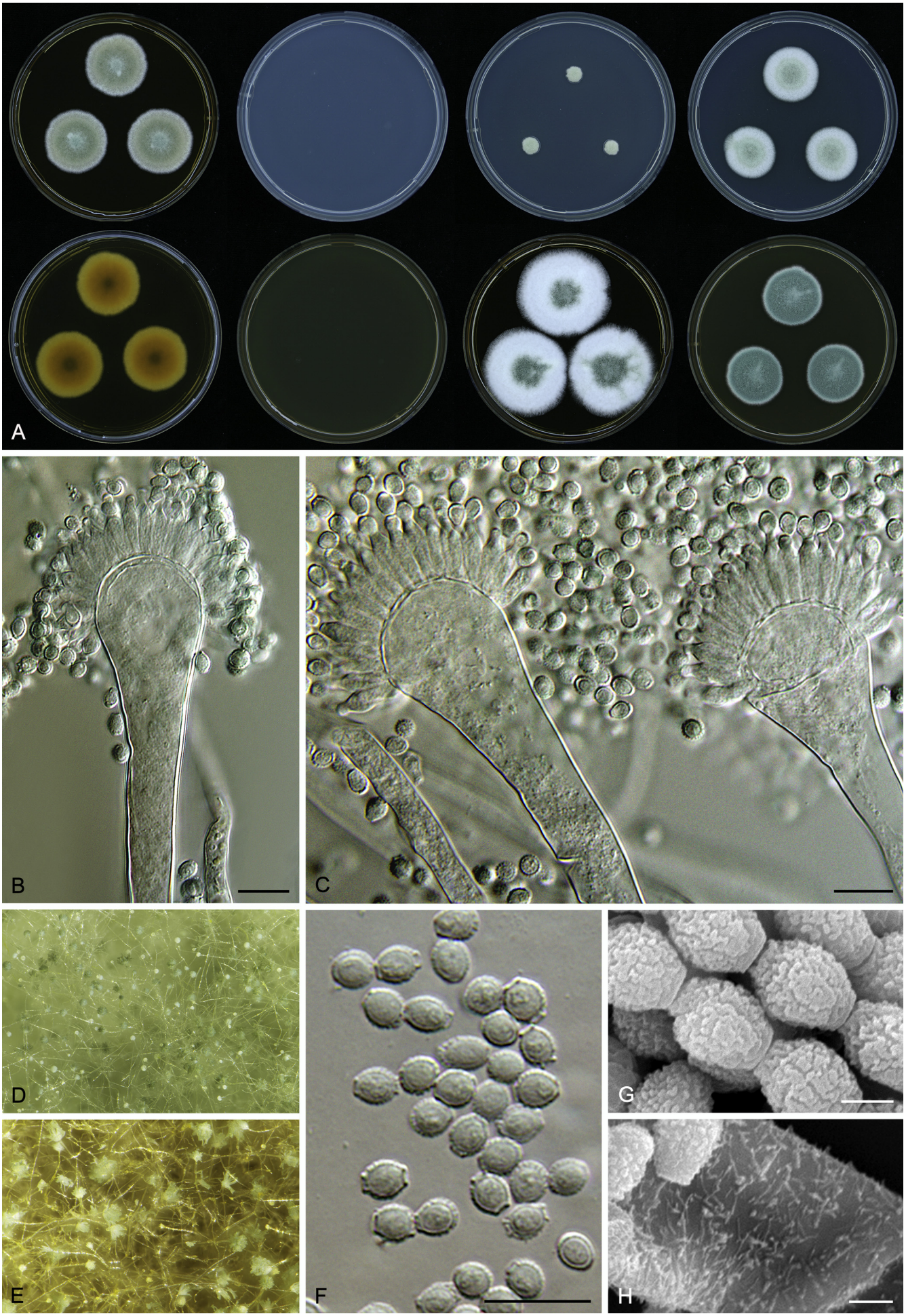


Fig. 16. *Aspergillus clavatophorus*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

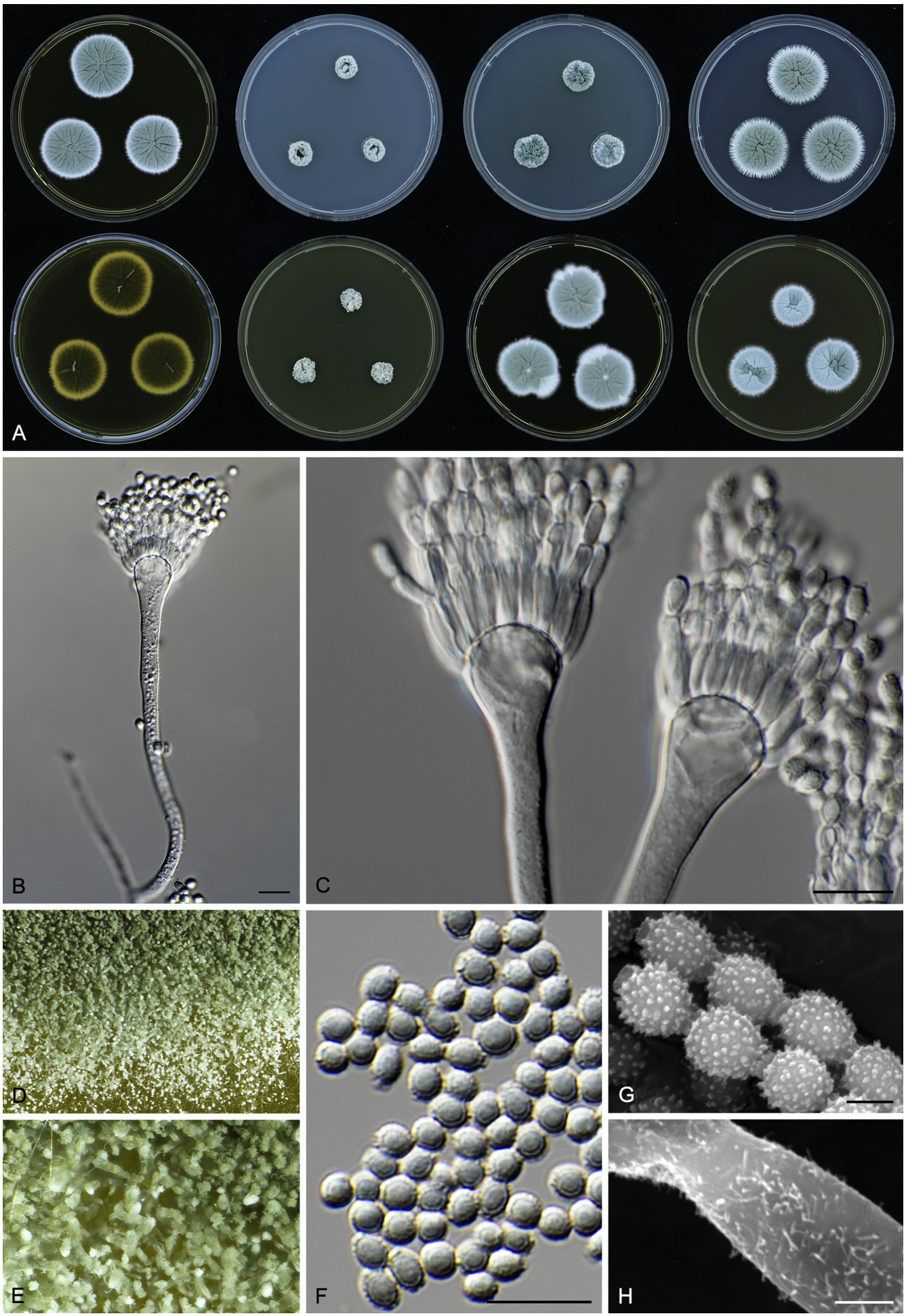


Fig. 17. *Aspergillus conicus*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

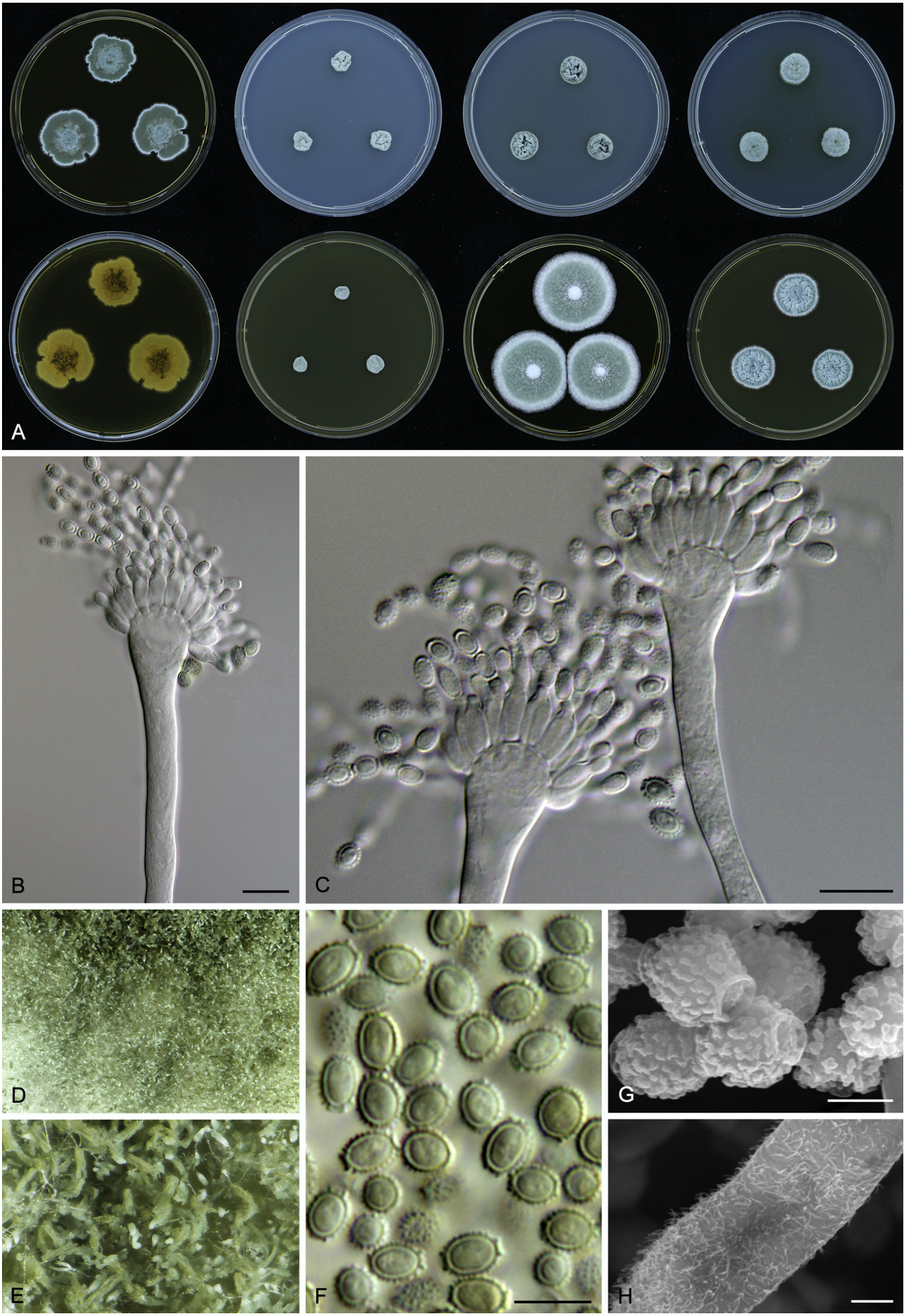


Fig. 18. *Aspergillus destruens*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

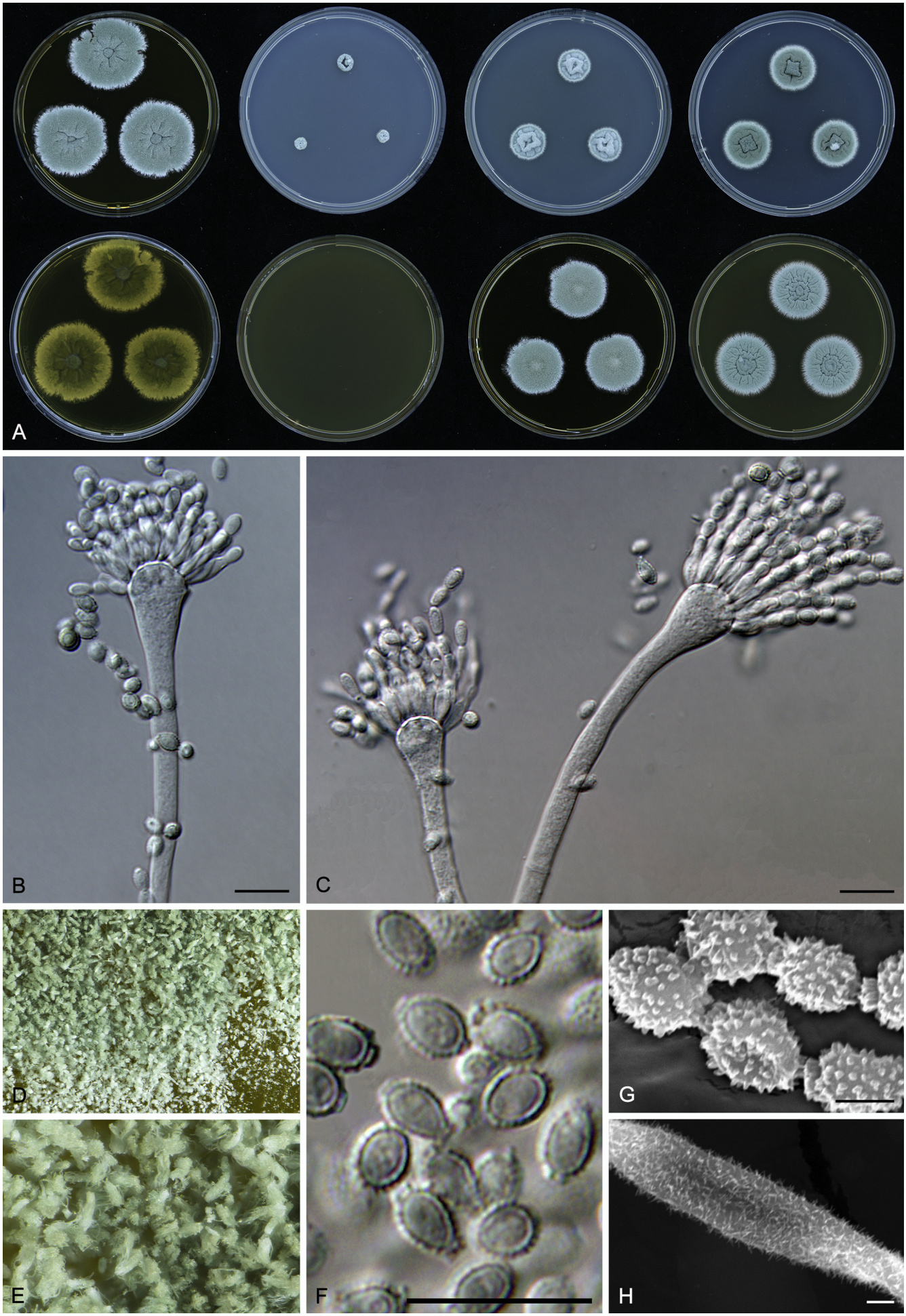


Fig. 19. *Aspergillus domesticus*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

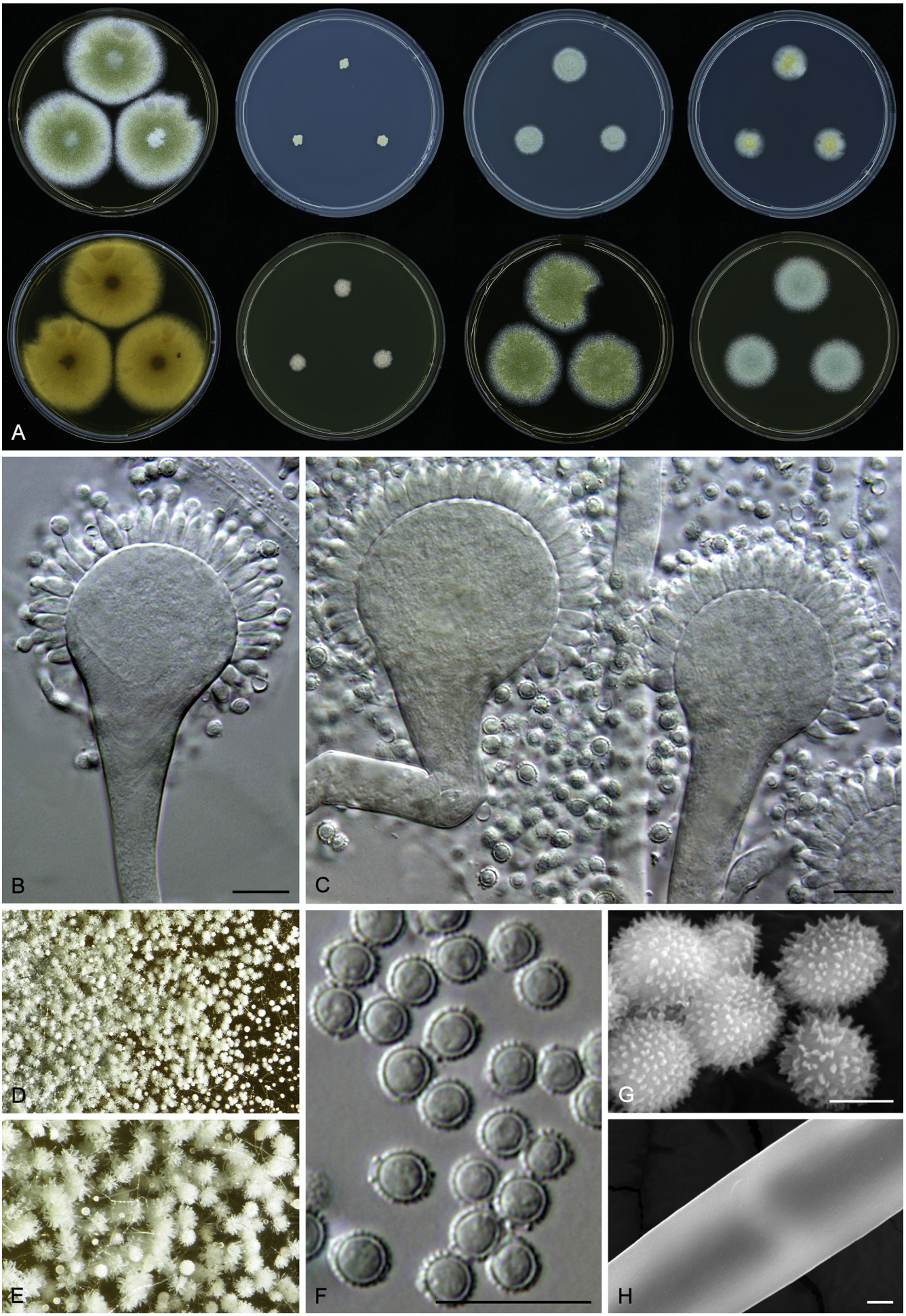


Fig. 20. *Aspergillus glabripes*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

centrally slightly raised; colony surface floccose, margin entire to delicately filiform; mycelial areas white; sporulation yellowish green (29B7); soluble pigment absent; exudate absent; reverse centrally yellowish grey (4D3) to yellowish white (4A2) in margins. DG18 25 °C, 14 d: umbonate; colony surface floccose, margin entire; mycelial areas yellowish white (4A2); sporulation pale green (29A3); soluble pigment absent; exudate absent; reverse centrally yellowish orange (4B7) to yellowish white (4A2) in margins. MEA 25 °C, 14 d: cerebriform; colony surface floccose, margin filiform; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally clay brownish orange (5C5) to pale orange (5A3) in margins. M60Y 25 °C, 14 d: flat; colony surface floccose; margin entire to delicately filiform; mycelial areas white; sporulation deep green (26D8) to yellowish green (29A8); soluble pigment absent; exudate absent; reverse centrally olive brown (4E5) to corn greyish yellow (4B5) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat; colony surface floccose, margin entire to delicately filiform; mycelial areas white; sporulation opal greyish green (25C6) to light green (28A5); soluble pigment absent; exudate absent; reverse centrally olive brown (2F6) to champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads radiate. Conidiophores uniseriate. Stipes smooth in SEM, 6–8 µm wide in the middle part, widening gradually toward the vesicle, with occasional septa. Vesicles globose, pyriform to subclavate, 16–22 µm wide. Phialides flask-shaped, 7–9 µm long, covering three quarters of the vesicle. Conidia borne as smooth cylinders, echinulate at maturity, aculeate in SEM, subglobose, connectives evident, 3.5–4.5 × 2–3 µm.

Distinguishing characters: See distinguishing characters of *A. vitricola*. *Aspergillus glabripes* can easily be differentiated from *A. vitricola* by its large vesicles that are most commonly globose or pyriform. Most *A. glabripes* isolates have a yellowish green colour of sporulation on M40Y and M60Y.

Additional materials examined: **Canada**, New Brunswick, Little Lepreau, house dust, 2015, collected by A. Walker, isolated by C.M. Visagie, KAS 6253. **France**, Brest, painting from Brittany (painter: A. Nozal; painting title: Saint-Briac, grève à marée basse; painting technique: pastel; and painter: B.M. Agüro; painting title: La vision de Saint Antoine de Padoue; painting technique: oil on canvas), 2015, isolated by F. Déniel, UBOCC-A-116021 = CCF 5467 = IBT 34625. **France**, brownies, 2014, isolated by F. Déniel, UBOCC-A-115046. **Trinidad & Tobago**, Macoya, office folder, 2014, isolated by Ž. Jurjević, CCF 5475 = EMSL No. 2463, CCF 5476 = EMSL No. 2464, CCF 5477 = EMSL No. 2465. **USA**, New Jersey, green fabric covered binders, import from China, 2014, isolated by Ž. Jurjević, CCF 5473 = EMSL No. 2442 = IBT 34626. **USA**, California, home air, 2011, isolated by Ž. Jurjević, CCF 5469 = EMSL No. 1483 = DTO 356-E5 = IBT 34519 = IBT 34821 = NRRL 66619. **USA**, Louisiana, library, front cover of log book, 2012, isolated by Ž. Jurjević, CCF 5470 = EMSL No. 1812. **USA**, Louisiana, library, book, 2012, isolated by Ž. Jurjević, CCF 5471 = EMSL No. 1813. **USA**, Idaho, home air, 2010, isolated by Ž. Jurjević, CCF 5468 = EMSL No. 1317. **USA**, South Carolina, Summerville, kitchen air, 2014, isolated by Ž. Jurjević, CCF 5472 = EMSL No. 2305.

Aspergillus gracilis Bainier, Bull. Soc. Mycol. Fr. 23: 92. 1907, emended description. MycoBank MB167554. Fig. 21.

Synonyms: *Aspergillus gracilis* var. *exiguus* Bainier & Sartory, Bull. Soc. Mycol. Fr. 28: 47. 1912.

Typus: **South Pacific**, gun-firing mechanism, unknown year of isolation, R. Emerson (neotype Herb. IMI 211393 designated by Samson & Gams (1985), culture ex-type NRRL 4962 = CBS 539.65 = DTO 351-H7 = CCF 5478 = ATCC 16906 = IMI 211393 = IBT 34817).

Colony diam, 14 d (*mm*): M40Y 30–42; CYA 0–8; CY20S 7–13; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 9–16; MEA No growth; M60Y 38–44; M60Y 30 °C 39–45; M60Y 37 °C No growth; MEA + 10 % NaCl 12–24.

Colony characters: M40Y 25 °C, 14 d: Colonies flat, wrinkled in the centre, surface velutinous; margin entire to delicately filiform; mycelial areas white; sporulation aquamarine greyish turquoise (24B3) to turquoise white (24A2); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2) to straw yellow (3B4), cream pale yellow (4A3) in margins. CYA 25 °C, 14 d: no growth to microcolonies. CY20S 25 °C, 14 d: crateriform; colony surface velutinous, margin filiform; mycelial areas white; sporulation turquoise grey (24C2); soluble pigment absent; exudate absent; reverse dull green (25D3) to pale orange (5A3) in margins. DG18 25 °C, 14 d: convex; colony surface floccose to velutinous, margin filiform; mycelial areas white; sporulation greyish turquoise (24D5); soluble pigment absent; exudate absent; reverse centrally yellowish grey (4B2) to orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: raised; colony surface granular to floccose in the centre; margin irregular, filiform; mycelial areas white; sporulation pale turquoise (24A3); soluble pigment absent; exudate absent; reverse centrally golden yellow (5B7) to champagne greyish yellow (4B4) in margins. MEA + 10 % NaCl 25 °C, 14 d: raised; colony surface velutinous, margin entire to irregular; mycelial areas white; sporulation pale turquoise (24A3); soluble pigment absent; exudate absent; reverse centrally cinnamon tan brown (6E6) to champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads loosely columnar, frequently twisted. Conidiophores uniseriate. Stipes smooth, densely covered by very long hairs in SEM, 3–5 µm wide in the middle part, with occasional septa. Vesicles spatulate to clavate, 6.5–9 µm wide. Phialides flask-shaped, densely covered by very long hairs in SEM, 7.5–9 µm long, covering up to two thirds of the vesicle. Conidia borne as smooth cylinders, echinulate at maturity, aculeate in SEM, subglobose or barrel-shaped, connectives evident, 3–4 × 2–3 µm.

Distinguishing characters: *Aspergillus gracilis* is most closely related to *A. pseudogracilis*. These two species can be distinguished from other species belonging to the *A. conicus* clade by their inability to grow on MEA and CYA at 25 °C (several strains of *A. gracilis* exhibit very restricted growth on CYA) and more rapid growth on M60Y at 25 °C and 30 °C. *Aspergillus pseudogracilis* can be distinguished from *A. gracilis* by its wider stipes, larger vesicles and longer phialides. Growth parameters of both species in the osmotic gradient are identical (see Fig. 12D).

Notes: *Aspergillus gracilis* var. *exiguus* was proposed based on slight differences in physiological characters and treated as synonym of *A. gracilis* by Raper & Fennell (1965). We could not obtain a living culture and no herbarium material is extant, resulting in us following conclusions of Raper & Fennell (1965).

Additional materials examined: **USA**, California, San Diego, child carrier, 2015, isolated by Ž. Jurjević, CCF 5479 = EMSL No. 2775 = DTO 356-F4 = IBT 34559, CCF 5480 = EMSL No. 2920, CCF 5481 = EMSL No. 2922, CCF 5482 = EMSL No. 2923 = IBT 34623, EMSL No. 2763, EMSL No. 2774.

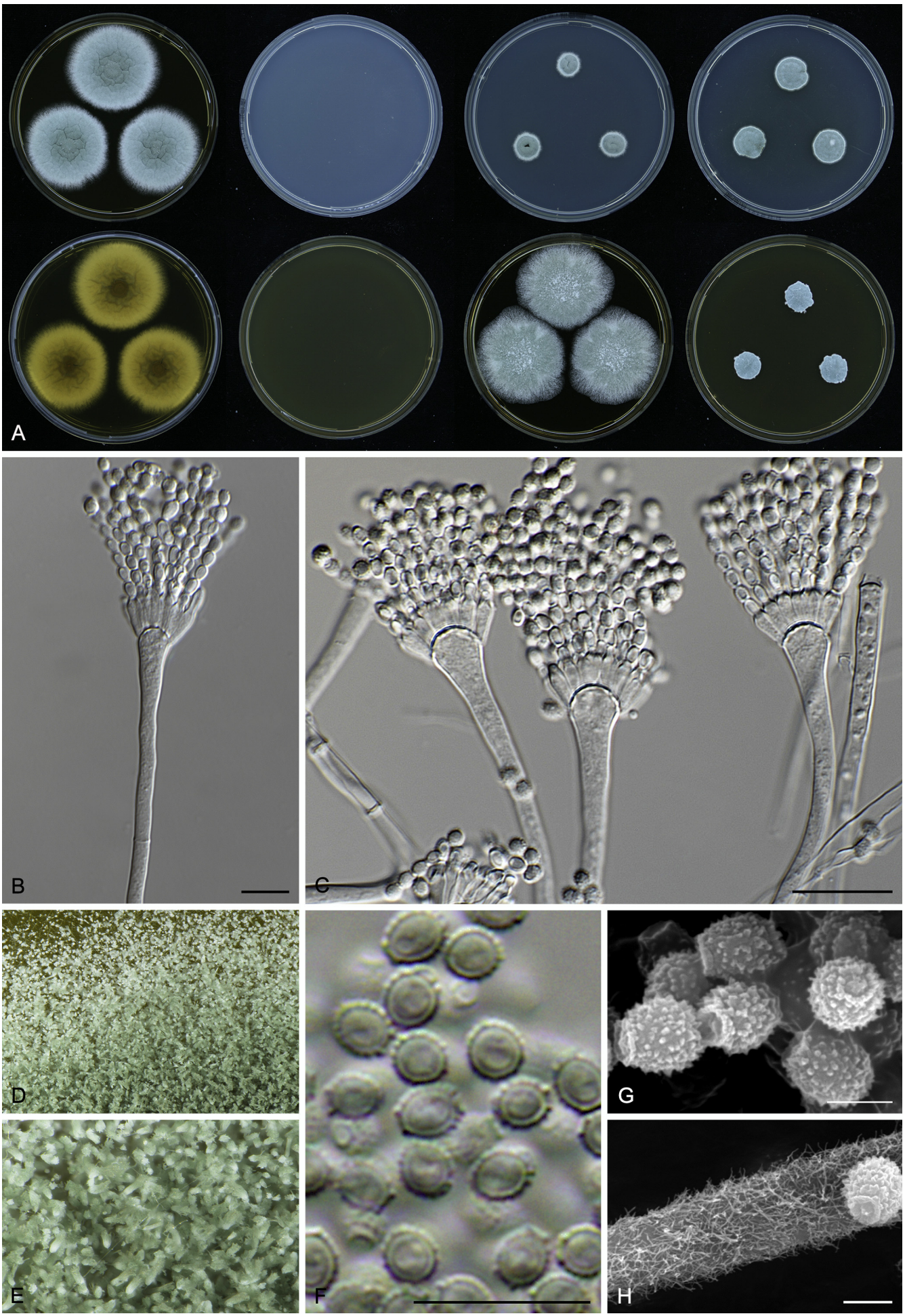


Fig. 21. *Aspergillus gracilis*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E**. Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

Aspergillus halophilicus C.M. Chr., Papav. & C.R. Benj., Mycologia 51: 636. 1959, emended description. MycoBank MB326633. Fig. 22.

Typus: **USA**, Minnesota, St. Paul, dried corn, 1957, isolated by C. M. Christensen [lectotype BPI 566153 designated by Samson & Gams (1985), culture ex-type NRRL 2739 = CCF 5687 = ATCC 16401 = CBS 12262 = IMI 211802 = IBT 34878].

Colony diam, 30 d (*mm*): CZA70S 10–12; no growth on all other tested media.

Colony characters: CZA70S 25 °C, 30 d: Colonies flat, surface floccose; margin filiform; mycelial areas hyaline to white; sporulation absent; ascomata produced after months of incubation, eurotium-like, hyaline to pale yellow; soluble pigment absent; exudate absent; reverse centrally greyish orange (6C6) to orange yellow (5A2) in margins.

Micromorphology: Ascomata maturing slowly within 1–2 mo on CZA70S at 25 °C, 100–150 µm diam, hyaline to pale yellow (3A3), asci globose to subglobose, 9–15 × 7–11 µm, ascospores hyaline, lenticular with two equatorial crests, convex surface finely roughened in the area neighbouring the equatorial region, with holes, 4.5–6.5 × 3.5–5 µm including crests.

Conidial state not observed in our cultures but (Christensen *et al.* 1959) observed it after 45 d on PDA + 20 % NaCl. Conidial heads at first globose, later becoming loosely radiate. Conidiophores uniseriate. Vesicles globose to subglobose or club-shaped, occasionally with one or two enlargements below the vesicle, 6–10 µm wide. Phialides flask-shaped, 6–14 µm long, covering only a portion of the top but occasionally up to three quarters of the vesicle, sometimes growing from the swellings below the vesicle. Conidia globose, elliptical, pyriform or irregular, spiny, hyaline to light blue-green, 6–11 × 4–6.5 µm.

Distinguishing characters: *Aspergillus halophilicus* can easily be distinguished from all other members of section *Restricti* by its eurotium-like sexual reproductive states and inability to grow on majority of tested media, including those with high sugar (M60Y) or salt (MEA + 10 % NaCl) content after 14 d.

Additional materials examined: **Italy**, Venice, book cover (swab), 2013, isolated by A. Micheluz, MUT 562. **Italy**, Lazio, Rome, Archivio Stampati del Vaticano, book cover (swab), 2010, isolated by M. Montanari, MUT 798. **Italy**, Marche, Loreto, Archivio della Casa Madre, book cover (swab), 2012, isolated by M. Montanari, MUT 1305. **Italy**, Piedmont, Torre Pellice, Archivio della Tavola Valdese, book cover (swab), 2012, isolated by M. Montanari, MUT 1307. **The Netherlands**, textile imported from unknown country, 2013, isolated by M. Meijer, DTO 271-F4 = CCF 5825 = IBT 34881.

Aspergillus hordei F. Sklenar, S.W. Peterson & Hubka, sp. nov. MycoBank MB818937. Fig. 23.

Etymology: Named after the isolation of species from barley (*Hordeum vulgare*).

Typus: **USA**, Minnesota, St. Paul, barley, 1957, isolated by G. C. Papavizas (holotype PRM 944446, isotype PRM 944447, culture ex-type NRRL 25825 = CCF 5483 = DTO 356-D3 = IBT 34539).

Colony diam, 14 d (*mm*): M40Y 25–29; CYA 0–8; CY20S (0)13–16; CY20S 30 °C (0)8–15; CY20S 37 °C No growth; DG18 13–26; MEA 0–5; M60Y 31–47; M60Y 30 °C 25–48; M60Y 37 °C 22–33; MEA + 10 % NaCl 26–29.

Colony characters: M40Y 25 °C, 14 d: Colonies umbonate, surface velutinous; margin entire; mycelial areas white; sporulation dark turquoise (24F8); soluble pigment absent; exudate absent; reverse centrally Cuba reddish brown (9E8), less commonly brownish red (8C7) to olive yellow (3C7), pale yellow (4A3) in margins. CYA 25 °C, 14 d: cerebriform; colony surface floccose, margin undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally orange grey (5B2) to orange white (5A2) in margins. CY20S 25 °C, 14 d: cerebriform to crateriform; colony surface velutinous, margin entire to delicately filiform; mycelial areas white; sporulation aquamarine greyish turquoise (24B3) to greyish turquoise (24D4); soluble pigment absent; exudate absent; reverse centrally aquamarine turquoise grey (24E2) to turquoise grey (24C2), orange white (5A2) in margins. DG18 25 °C, 14 d: crateriform; colony surface velutinous, margin entire to delicately filiform; mycelial areas white; sporulation aquamarine dark turquoise (24F8); soluble pigment greyish yellow (2B3); exudate absent; reverse centrally yellowish brown (5E8) to olive grey (2E2), champagne greyish yellow (4B4) to yellowish white (4A2) in margins. MEA 25 °C, 14 d: no growth to microcolonies. M60Y 25 °C, 14 d: flat; colony surface velutinous; margin entire; mycelial areas white; sporulation dark turquoise (24F7); soluble pigment absent; exudate absent; reverse centrally cinnamon brown (6D6) to pale yellow (4A3), greyish yellow (4C5) to pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat to slightly centrally raised; colony surface floccose to velutinose; margin entire; mycelial areas white; sporulation deep turquoise (24E8) to greyish turquoise (24B4); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2), greyish orange (5B4) to cream pale yellow (4A3) in margins.

Micromorphology: Conidial heads at first globose, later becoming loosely columnar. Conidiophores uniseriate. Stipes smooth, densely covered by short hairs in SEM, 4.5–6 µm wide in the middle part, with occasional septa. Vesicles spatulate, ellipsoidal, pyriform to subclavate, 12–14.5 µm wide. Phialides flask-shaped, densely covered by short hairs in SEM, 8.5–11 µm long, covering one to two thirds of the vesicle. Conidia borne smooth and ellipsoidal, becoming coarsely roughned, globose or barrel-shaped at maturity, lobate-reticulate in SEM, connectives evident, 3.5–5 × 2.5–4 µm.

Distinguishing characters: See distinguishing characters of *A. penicillioides*.

Additional materials examined: **Panama**, telescope reticle, 1945, collector unknown, NRRL 25812 = CCF 5658. **The Netherlands**, cigars, unknown year of isolation, isolated by M. Meijer, DTO 318-F5, DTO 318-F6, DTO 318-F7, DTO 321-F9, DTO 321-G1. **The Netherlands**, leather, imported from unknown country, 2013, isolated by J. Houbraken, DTO 281-A4 = IBT 34517. **USA**, Minnesota, St. Paul, barley, 1957, isolated by G. C. Papavizas, NRRL 25826 = CCF 5484. **USA**, Minnesota, St. Paul, insulating board, 1957, isolated by G. C. Papavizas, NRRL 25830 = CCF 5485 = IBT 34631.

Aspergillus infrequens F. Sklenar, S.W. Peterson & Hubka, sp. nov. Mycobank MB818938. Fig. 24.

Etymology: Referring to its rare occurrence.

Typus: **USA**, Illinois, Peoria, wheat, 1971, isolated by D. I. Fennell (holotype PRM 944449, isotype PRM 944450, culture ex-type NRRL 25868 = CCF 5486 = DTO 356-D6 = IBT 34524).

Colony diam, 14 d (*mm*): M40Y 21–22; CYA 4–5; CY20S 7–8; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 25–26; MEA No growth; M60Y 40–43; M60Y 30 °C 8–10; M60Y 37 °C No growth; MEA + 10 % NaCl 18–19.

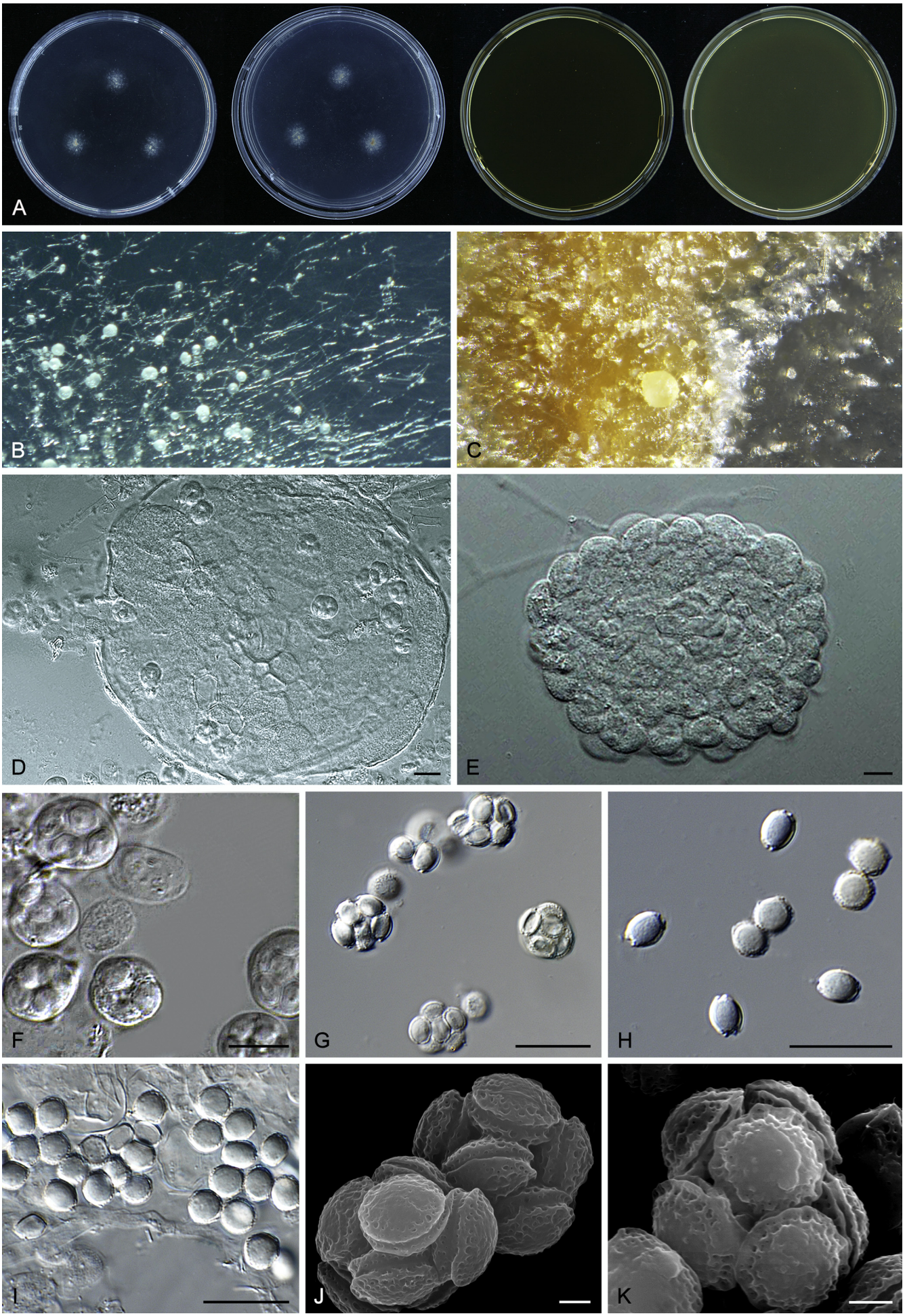


Fig. 22. *Aspergillus halophilicus*. **A.** Colonies after 14 d at 25 °C: left to right, obverse CZA70S, reverse CZA70S, M40Y and MEA + 10 % NaCl. **B–E.** Cleistothecia. **F, G.** Asci. **H, I.** Ascospores. **J, K.** SEM (ascospores). Scale bars: D–I = 10 µm; J, K = 2 µm.

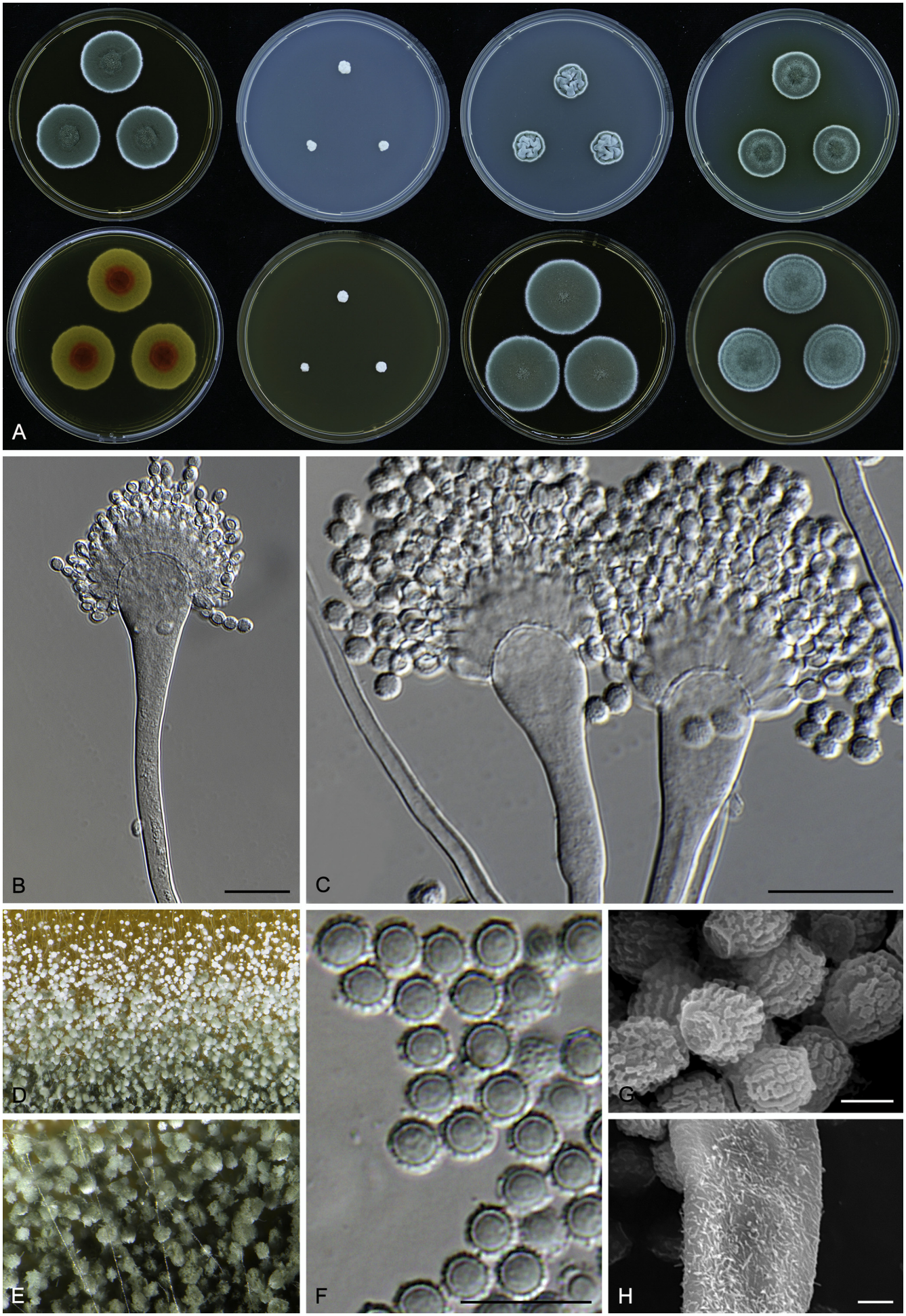


Fig. 23. *Aspergillus hordei*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

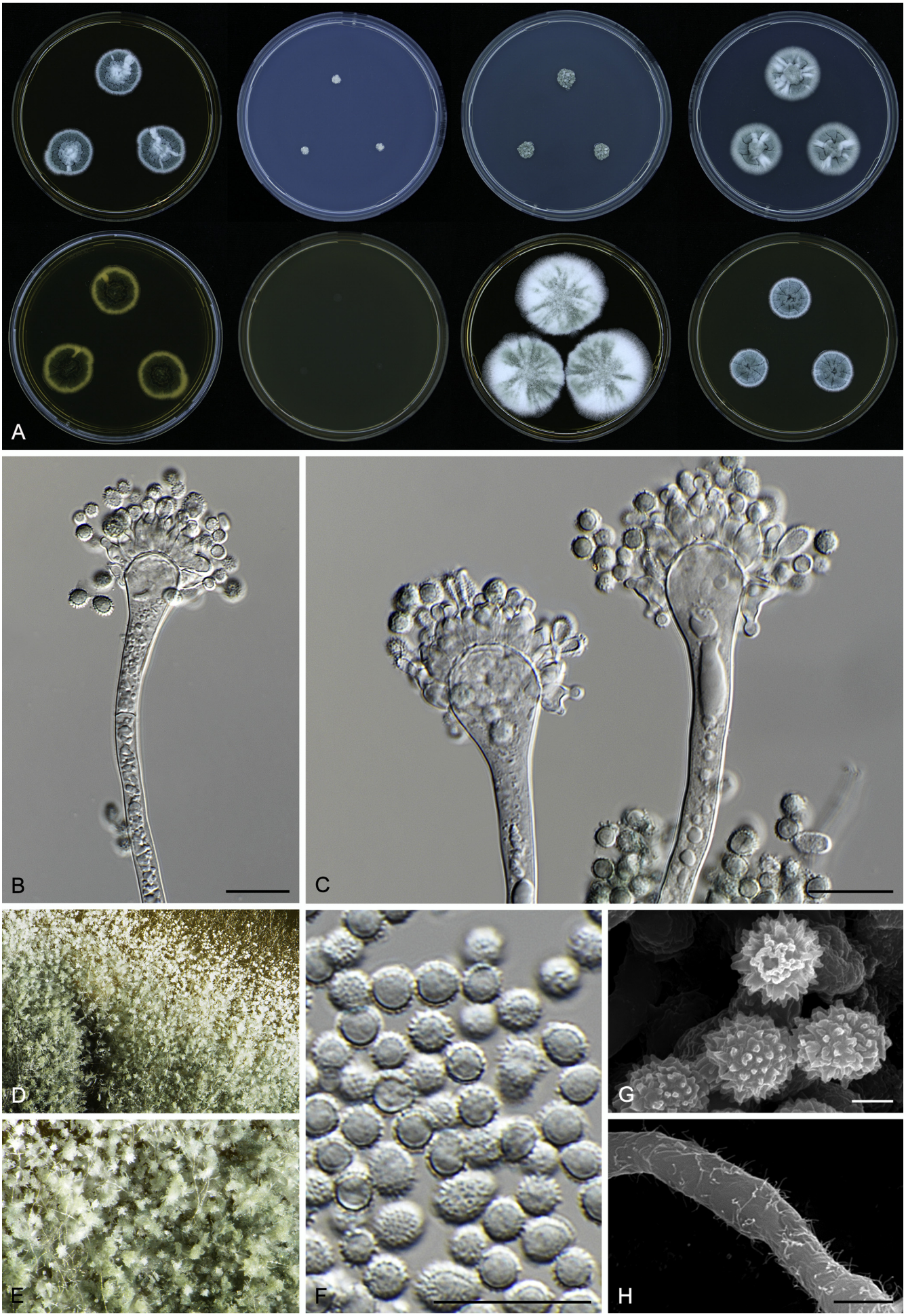


Fig. 24. *Aspergillus infrequens*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

Colony characters: M40Y 25 °C, 14 d: Colonies crateriform, wrinkled, surface floccose; margin filiform; mycelial areas white; sporulation pale turquoise (24A3) to dark turquoise (24F8); soluble pigment absent; exudate absent; reverse centrally dark green (30F8), olive grey (2F2) to dark green (30F8), champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: no growth to microcolonies. CY20S 25 °C, 14 d: crateriform, wrinkled; colony surface floccose, margin undulate; mycelial areas white; sporulation pale green (26A3) to pastel green (25A4); soluble pigment absent; exudate absent; reverse centrally turquoise grey (24D2) to greyish orange (5B3), orange white (5A2) in margins. DG18 25 °C, 14 d: umbonate, wrinkled; colony surface velutinous, margin filiform; mycelial areas white; sporulation pastel green (25A4) to greyish turquoise (water blue) (24C5); soluble pigment absent; exudate absent; reverse centrally dark turquoise (24F8) to greyish turquoise (24D5), orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: raised; colony surface powdery to cottony; margin filiform; mycelial areas white; sporulation greyish green (26B4); soluble pigment absent; exudate absent; reverse centrally olive (2E7) to mustard yellow (3B6), cream pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: crateriform, wrinkled; colony surface velutinous; margin filiform; mycelial areas white; sporulation light turquoise (24A4); soluble pigment absent; exudate absent; reverse centrally slate grey (3F2) to olive (3E7), champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads radiate. Conidiophores uniseriate. Stipes smooth, sparsely covered by bundles of long hairs in SEM, 6–7 µm wide in the middle part, non-septate. Vesicles pyriform, spatulate to clavate, (10–)13–17(–20) µm wide. Phialides flask-shaped, sparsely covered by long hairs in SEM, 8–10 µm long, covering one third to two thirds of the vesicle. Conidia borne smooth and ellipsoidal, becoming echinulate, subglobose, ovate or barrel-shaped at maturity, aculeate in SEM, commonly remaining in chains, connectives evident, 4–5 × 3–4 µm.

Distinguishing characters: *Aspergillus infrequens* is represented only by the ex-type isolate. It is phylogenetically distinct from other species in the *A. penicillioides* clade and resolves in the basal position. Although *A. infrequens* phenotypically resembles species from the *A. penicillioides* clade, the ornamentation of conidia in SEM is aculeate and atypical for the clade (this ornamentation is found in the *A. restrictus*, *A. conicus* and *A. vitricola* clades).

Aspergillus magnivesiculatus F. Sklenar, Zalar, Ž. Jurjević & Hubka, sp. nov. Mycobank MB818939. Fig. 25.

Etymology: Referring to its large vesicles.

Typus: **Japan**, Tokyo, katsuobushi, 1967, isolated by K. Yamada (holotype PRM 944444, isotype PRM 944445, culture ex-type NRRL 25866 = CCF 5488 = IBT 34816).

Colony diam, 14 d (*mm*): M40Y 27–35; CYA No growth; CY20S 11–17; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 20–26; MEA No growth; M60Y 47–60; M60Y 30 °C 40–50; M60Y 37 °C No growth; MEA + 10 % NaCl 27–34.

Colony characters: M40Y 25 °C, 14 d: Colonies raised to umbonate, surface floccose; margin entire to delicately filiform; mycelial areas centrally yellow (3A6) to white in margins; sporulation greyish green (26D5); soluble pigment absent; exudate absent; reverse centrally mahogany red (8E7), yellowish red (8B8) to pale yellow (4A3) in margins. CYA 25 °C, 14 d: no growth. CY20S 25 °C, 14 d: crateriform; colony surface velutinous, margin entire; mycelial areas white; sporulation greyish green (25B4) to greyish turquoise (24B4); soluble pigment absent; exudate absent; reverse centrally aquamarine turquoise grey (24D2) to orange white (5A2) in margins. DG18 25 °C, 14 d: umbonate; colony surface velutinous to floccose, margin entire to delicately filiform; mycelial areas centrally yellowish white (4A2) to white in margins; sporulation aquamarine greenish white (27A2); soluble pigment absent; exudate absent; reverse centrally corn greyish yellow (4B5) to yellowish white (4A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: raised; colony surface floccose to cottony; margin filiform; mycelial areas pastel yellow (3A4), pale yellow (4A3) or white; sporulation pale green (27A3) to white; soluble pigment absent; exudate absent; reverse centrally caramel brown (6C6), apricot yellow (5B6) to corn greyish yellow (4B5), pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat or centrally slightly raised; colony surface floccose to powdery; margin entire to delicately filiform; mycelial areas centraly light yellow (4A4) to white in margins; sporulation greyish green (26D5); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2) to golden brown (5D7), cream pale yellow (4A3) in margins.

Micromorphology: Conidial heads at first globose, later becoming loosely columnar to radiate. Conidiophores uniseriate. Stipes smooth, densely covered by short hairs in SEM, 5–7 µm wide in the middle part, non-septate. Vesicles pyriform, subclavate to clavate, 17–22 µm wide. Phialides flask-shaped, densely covered by short hairs in SEM, 9–11 µm long, covering two thirds to three quarters of the vesicle. Conidia borne smooth, ellipsoidal, coarsely roughned, ovate to barrel-shaped at maturity, tuberculate in SEM, connectives evident, 4.5–5.5 × 3.5–4.5 µm.

Distinguishing characters: Isolates of *A. magnivesiculatus* have the largest vesicles among species of the *A. penicillioides* clade; reverse colour on M40Y is usually mahogany red. See also distinguishing characters of *A. penicillioides*.

Additional materials examined: **Canada**, Nova Scotia, Wolfville, house dust, 2015, collected by A. Walker, isolated by C.M. Visagie, KAS 6089 = DTO 356-H3. **Canada**, Ontario, Ottawa, house dust, 2015, collected and isolated by C. M. Visagie, KAS 5655 = DTO 356-G8, KAS 5754 = DTO 356-G9. **Canada**, Ontario, Stittsville, house dust, 2014, collected by K. A. Seifert, isolated by C. M. Visagie, KAS 5623 = DTO 356-G7. **Japan**, Tokyo, katsuobushi, 1967, isolated by K. Yamada, NRRL 25867 = CCF 5660. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar and D.D. Graf, EXF-10353 = IBT 34284. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar and D.D. Graf, EXF-10377, EXF-10347 = IBT 34283, EXF-10356 = IBT 34279. **USA**, California, San Diego, child carrier, 2014, isolated by Ž. Jurjević, CCF 5490 = EMSL No. 2741. **USA**, California, San Diego, child carrier, 2015, isolated by Ž. Jurjević, CCF 5491 = EMSL No. 2918. **USA**, Idaho, home air, 2010, isolated by Ž. Jurjević, CCF 5489 = EMSL No. 1315 = DTO 356-E2 = IBT 34516. **USA**, Minnesota, St. Paul, dried corn, 1954, isolated by C. M. Christensen, NRRL 25821 = CCF 5487.

Aspergillus pachycaulis F. Sklenar, S.W. Peterson, Ž. Jurjević & Hubka, sp. nov. MycoBank MB823048. Fig. 26.

Etymology: Referring to the wide conidiophore stipes, especially compared to the closely related *A. caesiellus*.

Typus: **USA**, District of Columbia, Washington, unknown substrate, 1956, isolated by H. E. Wheeler (holotype PRM 944432,

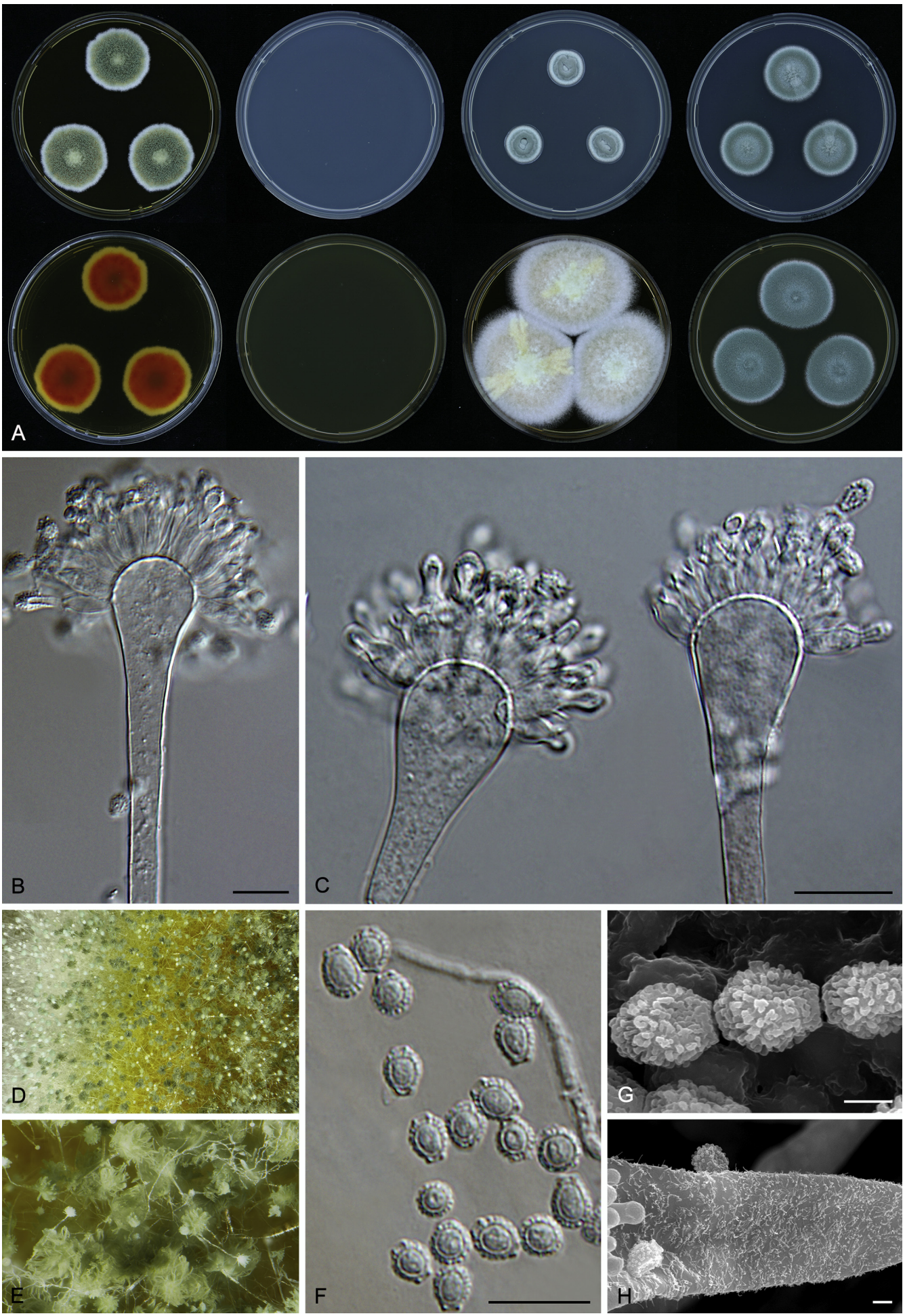


Fig. 25. *Aspergillus magnivesiculatus*. **A.** Colonies: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

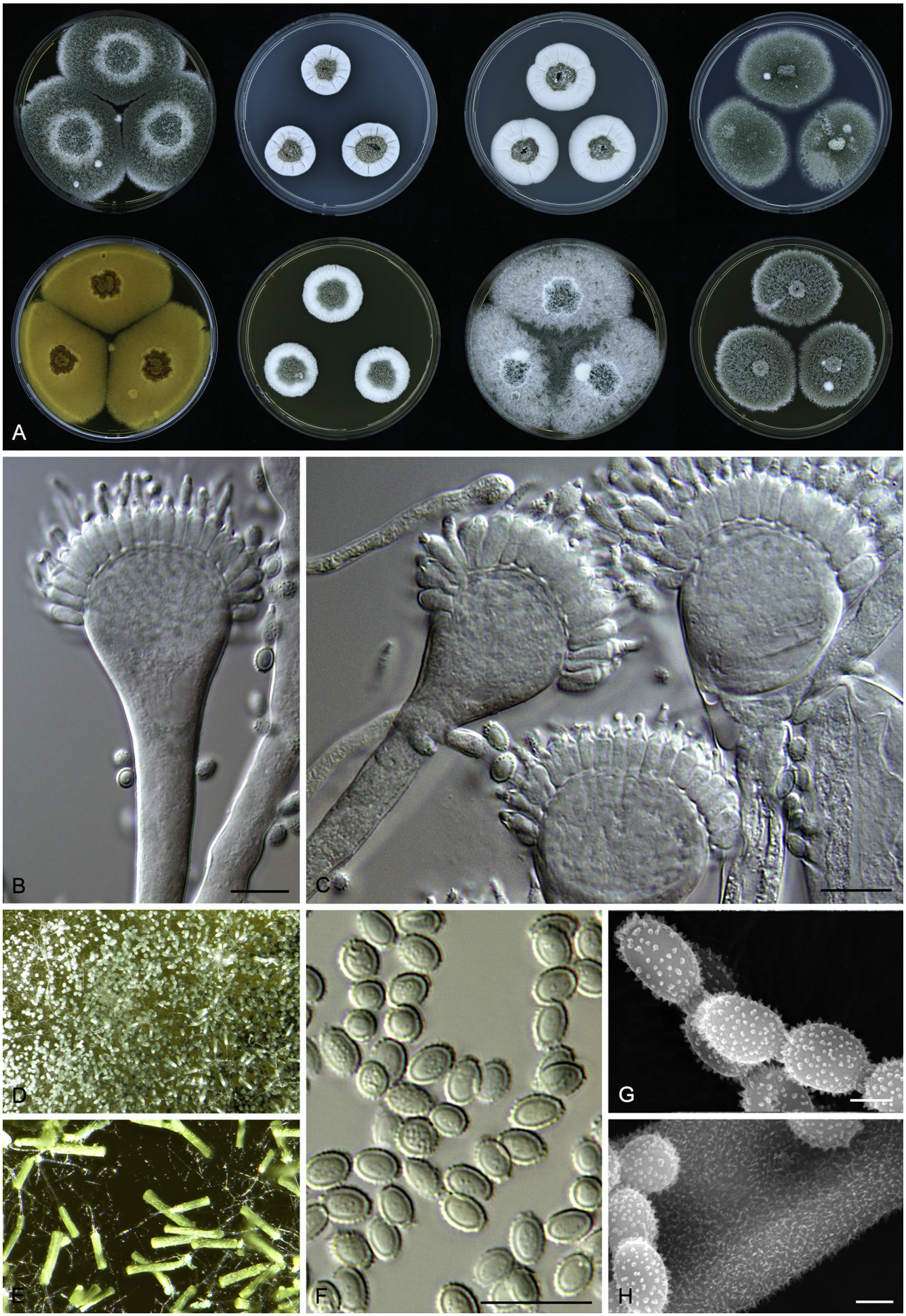


Fig. 26. *Aspergillus pachycaulis*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

isotype PRM 944433, culture ex-type NRRL 25824 = CCF 5492 = DTO 356-D2 = IBT 34521 = IBT 34812).

Colony diam, 14 d (*mm*): M40Y 20–60; CYA 11–26; CY20S 15–40; CY20S 30 °C 22–36; CY20S 37 °C (0)8–10; DG18 35–48; MEA 21–31; M60Y 25–60; M60Y 30 °C 36–60; M60Y 37 °C 38–60; MEA + 10 % NaCl 27–43.

Colony characters: M40Y 25 °C, 14 d: Colonies raised, surface powdery to floccose; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24C4); soluble pigment absent; exudate absent; reverse centrally brownish yellow (5C8) to light yellow (4A4) in margins. CYA 25 °C, 14 d: centrally raised, radially wrinkled; colony surface lanose to floccose, margin entire; mycelial areas white; sporulation greyish green (25C3); soluble pigment absent; exudate absent; reverse centrally deep blue (21D8) to yellowish white (2A2) in margins. CY20S 25 °C, 14 d: with central depression; colony surface lanose to floccose; margin entire; mycelial areas white; sporulation dull green (25D3); soluble pigment absent; exudate absent; reverse centrally greyish brown nougat (5D3) to orange white (5A2) in margins. DG18 25 °C, 14 d: flat or umbonate; colony surface powdery to floccose, margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (water blue) (24D5); soluble pigment absent; exudate absent; reverse milk white (1A2). MEA 25 °C, 14 d: centrally raised; colony surface lanose to floccose, margin entire; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally reddish golden (6C7) to pale orange (5A3) in margins. M60Y 25 °C, 14 d: raised; colony surface lanose to filamentous; margin entire to delicately filiform; mycelial areas white; sporulation dull green (27D4); soluble pigment absent; exudate absent; reverse centrally Pompeian yellow (5C6) to pastel yellow (3A4) in margins. MEA + 10 % NaCl 25 °C, 14 d: umbonate; colony surface powdery; margin irregularly filiform; mycelial areas white; sporulation greyish blue (23C5); soluble pigment absent; exudate absent; reverse centrally greyish orange (5B4) to light yellow (4A4) in margins.

Micromorphology: Conidial heads columnar, frequently sinuous. Conidiophores uniseriate. Stipes smooth, densely covered by short hairs in SEM, occasionally with septa, 7–8 µm wide. Vesicles pyriform to subclavate, 16–20 µm wide. Phialides flask-shaped, densely covered by short hairs in SEM, 8–9 µm long, covering one to two thirds of the vesicle. Conidia borne as smooth cylinders, at maturity ellipsoidal to ovate, aculeate in SEM, connectives evident, 4–5 × 2.5–3.5 µm.

Distinguishing characters: See distinguishing characters of *A. restrictus*. *Aspergillus pachycaulis* has wider stipes and larger vesicles compared to *A. caesiellus* and *A. restrictus*. It also grows slightly faster on MEA and some isolates can grow on CY20S at 37 °C (exception throughout the whole sect. *Restricti*).

Additional materials examined: **Canada**, Ontario, Ottawa, unknown substrate, isolated by J. Bissett, DAOMC 226750 = KAS 7748. **USA**, California, home air, 2005, isolated by Ž. Jurjević, CCF 5493 = EMSL No. 2310 = DTO 356-E6 = IBT 34536. **Unknown locality of isolation**, unknown substrate, unknown year of isolation, collector unknown, DTO 066-I6.

Aspergillus penicillioides Speg., Revista Fac. Agron. Univ. Nac. La Plata 2: 245. 1896, emended description. MycoBank MB309234. Fig. 27.

Typus: **Brazil**, Recife, isolated from human cutaneous disease, 1958, D. Borelli (neotype Herb. IMI 211392 designated by Samson & Gams (1985), culture ex-type NRRL 4548 = CBS 540.65 = ATCC 16910 = IMI 211342 = DTO 207-I7 = CCF 5494 = IBT 34627).

Colony diam, 14 d (*mm*): M40Y 27–43; CYA 7–9; CY20S 11–20; CY20S 30 °C 11–15; CY20S 37 °C No growth; DG18 17–36; MEA 5–9; M60Y 28–59; M60Y 30 °C 40–60; M60Y 37 °C 17–45; MEA + 10 % NaCl 24–37.

Colony characters: M40Y 25 °C, 14 d: Colonies raised to umbonate, surface powdery; margin entire to delicately filiform; mycelial areas white; sporulation deep green (25E8); soluble pigment absent; exudate absent; reverse centrally coffee brown (5F7) to yellowish brown (5E8), golden greyish yellow (4C6) to orange white (5A2) in margins. CYA 25 °C, 14 d: convex, wrinkled; colony surface velutinous, margin undulate; mycelial areas white; no sporulation; soluble pigment absent; exudate absent; reverse centrally greyish orange (5B3) to cream orange white (5A2) in margins. CY20S 25 °C, 14 d: crateriform; colony surface velutinous, margin filiform; mycelial areas white; sporulation pale green (25A3); soluble pigment absent; exudate absent; reverse centrally greyish yellow (2B5) to olive grey (2F2), greyish orange (5B3) to orange white (5A2) in margins. DG18 25 °C, 14 d: umbonate to convex; colony surface floccose, margin filiform; mycelial areas white; sporulation aquamarine light turquoise (24A4); soluble pigment greyish yellow (2B3); exudate absent; reverse centrally olive yellow (2D6) to yellowish white (4A2) in margins. MEA 25 °C, 14 d: crateriform; colony surface velutinous, margin entire; no sporulation; soluble pigment absent; exudate absent; reverse centrally caramel brown (6C6) to pale orange (5A3) in margins. M60Y 25 °C, 14 d: raised; colony surface cottony; margin entire to delicately filiform; mycelial areas white; sporulation greyish green (25D7); soluble pigment absent; exudate absent; reverse centrally dark brown (6F6) to golden greyish yellow (4C6), pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: centrally raised; colony surface floccose; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24C6); soluble pigment absent; exudate absent; reverse centrally olive brown (4F5) to yellowish orange (4B7), cream pale yellow (4A3) in margins.

Micromorphology: Conidial heads at first globose, later becoming loosely columnar. Conidiophores uniseriate. Stipes smooth, sparsely covered by short hairs in SEM, 4–6 µm wide in the middle part, widening gradually toward the vesicle, non-septate. Vesicles pyriform, spatulate to clavate, 10–18 µm wide. Phialides flask-shaped, sparsely covered by short hairs in SEM, 8–10 µm long, covering upper half to two thirds of the vesicle. Conidia borne smooth and ellipsoidal, later becoming coarsely roughened, subglobose or barrel-shaped, tuberculate in SEM, commonly remaining in chains, connectives evident, 3.5–4.5 × 2.5–3.5 µm.

Distinguishing characters: All species belonging to the *A. penicillioides* clade are morphologically very similar and particularly *A. penicillioides* and *A. hordei* share almost identical morphologies. The latter two species can be distinguished from others in the clade by their ability to grow on CY20S at 30 °C, production of greyish yellow soluble pigment when cultivated on DG18, narrow stipes, small vesicles and loosely columnar rather then loosely radiate conidial heads. In contrast to most *A. hordei* isolates, *A. penicillioides* always grows on MEA and CYA. Conidia of *A. penicillioides* are tuberculate in SEM, but lobate-reticulate in *A. hordei*.

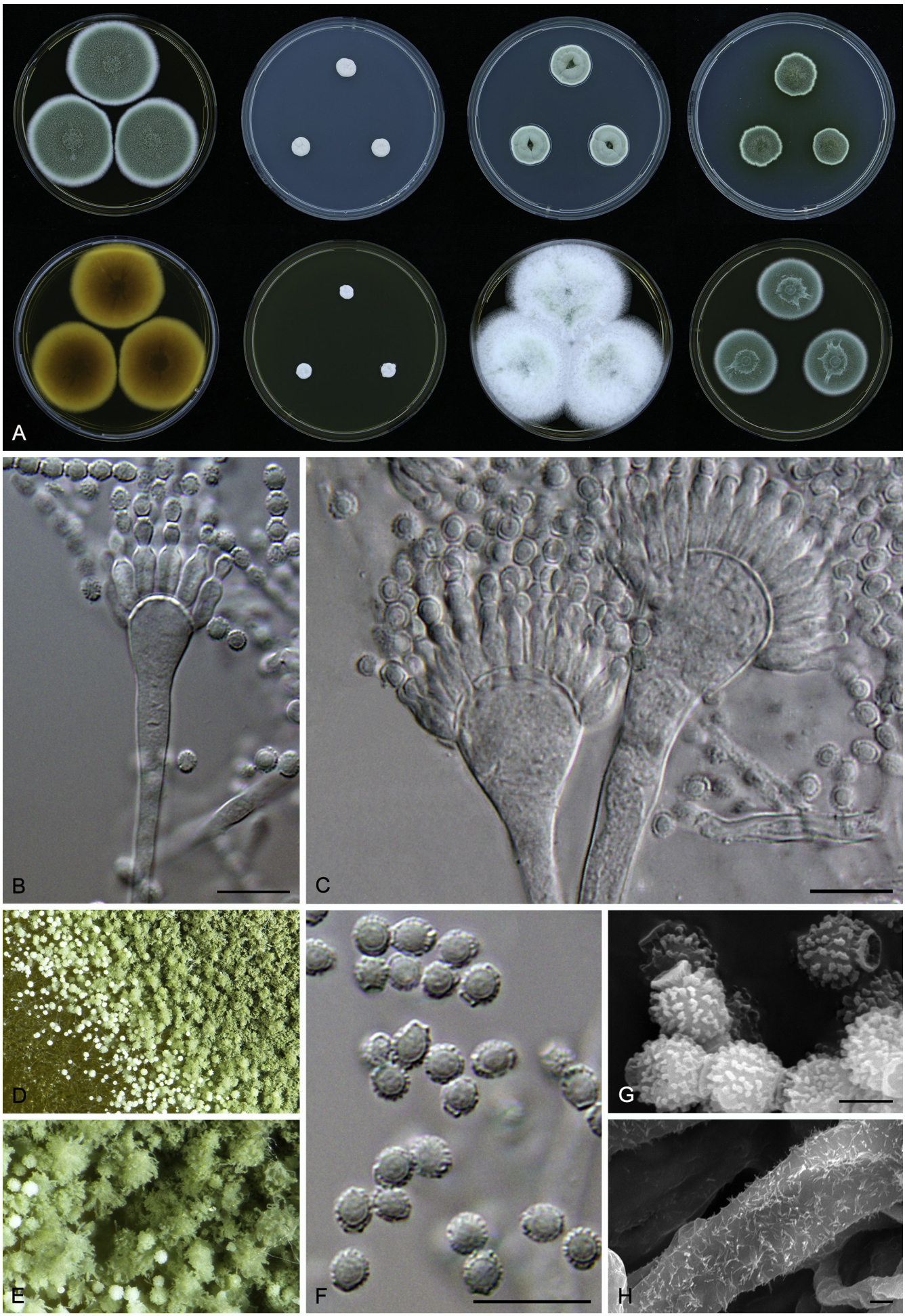


Fig. 27. *Aspergillus penicillioides*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

Additional materials examined: **Belgium**, Brussels, indoor air (air-conditioned office), 1985, collector unknown, IHEM 2476. **Brazil**, Recife, human cutaneous disease isolate, 1958, collector unknown, NRRL 4550 = CCF 5495, NRRL 4553 = CCF 5496. **Canada**, Ontario, Ottawa, house dust, 2015, collected by N. Yilmaz, isolated by C. M. Visagie, KAS 7745, KAS 7746. **Czech Republic**, Prague, sweet roll with chocolate glaze, 2002, isolated by H. Růžičková, CCF 3282. **Czech Republic**, Zlín, leather shoe, 1990, isolated by T. Skálová, CCF 2666. **France**, seeds of cereal, 1984, collector unknown, IHEM 2330. **France**, French madeleines, 2013, isolated by F. Déniel, UBOCC-A-115042 = CBS 140430 = DTO 334-E1. **Germany**, unknown substrate, unknown year of isolation, collector unknown, DTO 334-A9. **Indonesia**, peanuts, 2008, isolated by E.S. Rahayu and J. Houbraken, DTO 063-G8. **Micronesia**, house dust, unknown year of isolation, isolated by C. M. Visagie, DTO 267-A4 = IBT 34614. **Micronesia**, house dust, 2009, collected by W. Law, isolated by E. Whitfield and K. Mwange, DTO 267-A9 = CCF 5664. **The Netherlands**, leather imported from unknown country, 2013, isolated by J. Houbraken, DTO 281-A7. **The Netherlands**, Zwartewaal, air, 2012, isolated by M. Meijer, 231-A7. **The Netherlands**, flour imported from unknown country, 2005, isolated by J. Houbraken, DTO 011-C4. **USA**, Illinois, O'fallon, baseball gloves in store, 2014, isolated by Ž. Jurjević, CCF 5500 = EMSL No. 2651 = IBT 34630. **USA**, New Jersey, green fabric covered binders, import from China, 2014, isolated by Ž. Jurjević, CCF 5499 = EMSL No. 2441, CCF 5497 = EMSL No. 2430 = IBT 34628, CCF 5498 = EMSL No. 2440 = DTO 356-E7 = IBT 34815. **USA**, North Carolina, Durham, unknown substrate, 1952, isolated by J. L. Miranda, NRRL 25816 = CCF 5661. **USA**, Minnesota, St. Paul, peas, 1957, isolated by G. C. Papavizas, NRRL 25834 = CCF 5659. **USA**, Minnesota, St. Paul, wheat, 1957, isolated by G. C. Papavizas, NRRL 25835. **USA**, California, San Diego, child carrier, 2015, isolated by Ž. Jurjević, CCF 5501 = EMSL No. 2749 = IBT 34629, CCF 5502 = EMSL No. 2900, CCF 5503 = EMSL No. 2909, EMSL No. 2747, EMSL No. 2777, EMSL No. 2766, EMSL No. 2786, EMSL No. 2760, EMSL No. 2757, EMSL No. 2904, EMSL No. 2898. **USA**, Maryland, Bethesda, archival cabinet, 2015, isolated by Ž. Jurjević, CCF 5504 = EMSL No. 3264. **USA**, New York, blood sample, 1951, isolated by A. Howell, NRRL 25879. **USA**, Minnesota, St. Paul, dried corn, 1954, isolated by C. M. Christensen, NRRL 25820, NRRL 25822. **Unknown locality of isolation**, unknown substrate, unknown year of isolation, collector unknown, DTO 008-H4. **Unknown locality of isolation**, unknown substrate, unknown year of isolation, collector unknown, NRRL 25870.

Aspergillus pseudogracilis F. Sklenar, Ž. Jurjević & Hubka, sp. nov. MycoBank MB818932. Fig. 28.

Etymology: Referring to its close phylogenetic and phenotypic relatedness to *A. gracillis.*

Typus: **USA**, California, San Diego, child carrier, 2015, isolated by Ž. Jurjević (holotype PRM 944434, isotype PRM 944435, culture ex-type CCF 5505 = EMSL No. 2765 = DTO 356-F3 = NRRL 66620 = IBT 34813).

Colony diam, 14 d (*mm*): M40Y 42–45; CYA No growth; CY20S 7–8; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 20–22; MEA No growth; M60Y 52–53; M60Y 30 °C 43–45; M60Y 37 °C No growth; MEA + 10 % NaCl 25–26.

Colony characters: M40Y 25 °C, 14 d: Colonies raised, surface floccose; margin entire to delicately filiform; mycelial areas white; sporulation light turquoise (24A4); soluble pigment absent; exudate absent; reverse centrally cinnamon brown (6D6) to champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: no growth to microcolonies. CY20S 25 °C, 14 d: convex; colony surface floccose to cottony, margin entire to delicately filiform; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally greyish orange (5B3) to yellowish white (4A2) in margins. DG18 25 °C, 14 d: centrally raised; colony surface floccose, margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24B4); soluble pigment absent; exudate absent; reverse centrally turquoise grey (24C2) to orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: raised; colony surface floccose; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24B4); soluble pigment absent; exudate absent; reverse champagne greyish yellow (4B4). MEA + 10 % NaCl 25 °C, 14 d: flat, wrinkled; colony surface floccose, margin entire to delicately filiform; mycelial areas white; sporulation pale turquoise (24A3); soluble pigment absent; exudate absent; reverse centrally light brown (5D6) to champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads loosely columnar, frequently twisted. Conidiophores uniseriate. Stipes smooth, densely covered by long hairs in SEM, 4–6 µm wide in the middle part, widening gradually toward the vesicle, septate. Vesicles spatulate to clavate, 9–13 µm wide. Phialides flask-shaped, densely covered by long hairs in SEM, 9–10.5 µm long, covering two thirds of the vesicle. Conidia borne as smooth cylinders, echinulate at maturity, aculeate in SEM, subglobose or barrel-shaped, connectives evident, 3–4 × 2–3 µm.

Distinguishing characters: See distinguishing characters of *A. conicus* and *A. gracilis*.

Aspergillus restrictus G. Sm., J. Textile Inst. 22: T115. 1931, emended description. MycoBank MB276290. Fig. 29.

Synonyms: *Penicillium fuscoflavum* S. Abe, J. Gen. Appl. Microbiol., Tokyo 2: 64. 1956.
Aspergillus restrictus var. *B* G. Sm., J. Textile Inst. 22: T115. 1931.

Typus: **United Kingdom**, cloth, 1928, isolated by G. Smith [neotype Herb. IMI 16267 designated by Samson & Gams (1985) and modified by Pitt & Samson (1993), culture ex-type NRRL 154 = CBS 117.33 = CBS 541.65 = DTO 079-B2 = ATCC 16912 = IHEM 3920 = IMI 16267= CCF 5506 = IBT 34615].

Colony diam, 14 d (*mm*): M40Y 42–53; CYA 12–16; CY20S 24–29; CY20S 30 °C 12–25; CY20S 37 °C No growth; DG18 28–33; MEA 5–13; M60Y 38–50; M60Y 30 °C 45–55; M60Y 37 °C 20–40; MEA + 10 % NaCl 33–40.

Colony characters: M40Y 25 °C, 14 d: Colonies flat, surface powdery, filamentous in margins; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24C4); soluble pigment absent; exudate absent; reverse centrally cinnamon brown (6D6) to sand greyish yellow (4B3) in margins. CYA 25 °C, 14 d: centrally raised, irregulary wrinkled; colony surface floccose, margin entire; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally dull blue (23E5) to orange white (5A2) in margins. CY20S 25 °C, 14 d: umbonate with concentric ring pattern; colony surface coarsely granular in the centre, velvety in margins; margin entire to delicately filiform; mycelial areas white; sporulation deep green (29E8) to light turquoise (25A4); soluble pigment absent; exudate absent; reverse centrally dull blue (23E5) to orange white (5A2) in margins. DG18 25 °C, 14 d: umbonate; colony surface powdery, margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24D4); soluble pigment absent; exudate absent; reverse centrally brownish orange (6C3) to orange white (5A2) in margins. MEA 25 °C, 14 d: convex, radially wrinkled; colony surface floccose, margin entire; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally light brown (7D4) to flesh greyish orange (6B3) in margins. M60Y 25 °C, 14 d: flat; colony surface powdery; margin filiform; mycelial areas white; sporulation greyish turquoise (24C4); soluble pigment absent; exudate absent; reverse light yellow (4C4). MEA + 10 %

Fig. 28. *Aspergillus pseudogracilis*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

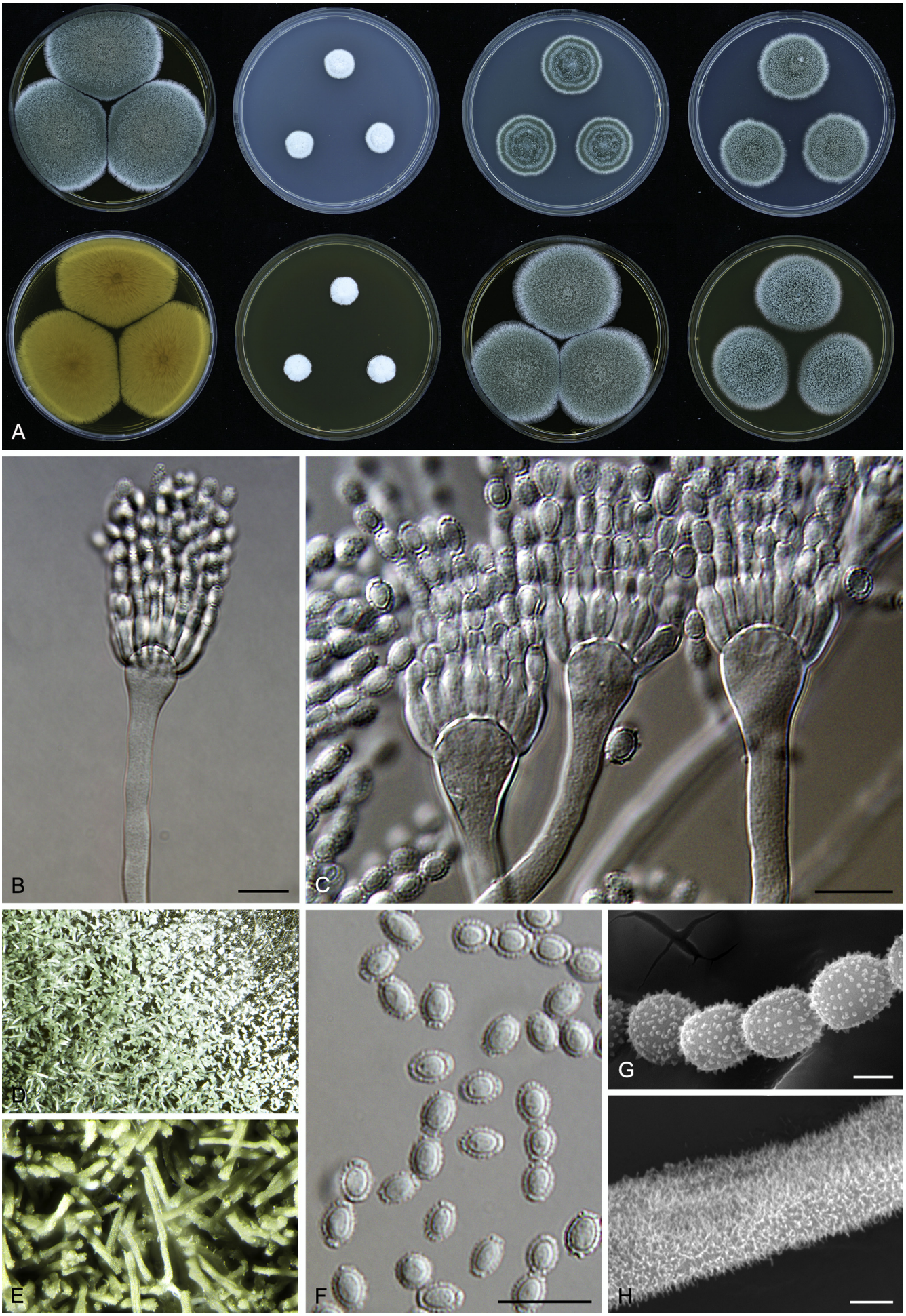


Fig. 29. *Aspergillus restrictus*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

NaCl 25 °C, 14 d: flat; colony surface powdery, filamentous in margins; margin entire to delicately filamentous; mycelial areas white; sporulation greyish turquoise (24C4); soluble pigment absent; exudate absent; reverse centrally brownish grey (8E2) to champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads columnar, frequently sinuous. Conidiophores uniseriate. Stipes smooth, densely covered by long hairs in SEM, non-septate, 4–5.5 µm wide. Vesicles pyriform to clavate, 9–12 µm in diameter. Phialides flask-shaped, densely covered by long hairs in SEM, 8–9 µm long, covering one third to one half of the vesicle. Conidia borne as smooth cylinders in long appressed columns, at maturity ellipsoidal to ovate, commonly remaining in chains, connectives evident, coarsely roughned to echinulate, aculeate in SEM, 3.5–4.5 × 2.5–3 µm.

Distinguishing characters: *Aspergillus restrictus* can be distinguished from *A. caesiellus* and *A. pachycaulis* by smaller vesicles and narrower stipes, slower growth on M40Y and M60Y and no sporulation on CYA. Osmotic gradient growth curves of these three species differ mainly on MEA, where *A. pachycaulis* grows slowest on MEA + 10 % NaCl and MEA + 15 % NaCl where *A. restrictus* grows fastest (see Fig. 12A).

Taxonomic notes: *Penicillium fuscoflavum* (ex-type strain NRRL 4783) and *A. restrictus* var. B (represented by NRRL 154) are taxa conspecific with *A. restrictus*, based on phylogenetic analysis (Peterson 2008, this study – see Fig. 7).

Additional materials examined: **Belgium**, Antwerp, dust from mattress, 1984, collector unknown, IHEM 2121. **Belgium**, Estinnes-au-Mont, indoor air, 1981, collector unknown, IHEM 818. **Belgium**, Brussels, indoor air (air-conditioned office), 1985, collector unknown, IHEM 2477. **Belgium**, Brussels, indoor air (air-conditioned office), 1984, collector unknown, IHEM 2373. **Bermuda**, home air, 2010, isolated by Ž. Jurjević, CCF 5507 = EMSL No. 1379 = IBT 34618. **Canada**, Ontario, Oshawa, maple syrup, unknown year of isolation, isolated by Samantha Frasz, DAOMC 250090 = KAS 7744. **Czech Republic**, Prague, sclerotium of fungus *Corallocytostroma ornicopreoides* imported from Australia, 2003, isolated by A. Kubátová, CCF 3364 = IBT 34619. **Indonesia**, corn, unknown year of isolation, collector unknown, DTO 065-C7 = IBT 34541. **Indonesia**, dust, unknown year of isolation, collector unknown, DTO 236-I7, DTO 237-A2, DTO 236-I8. **USA**, Maryland, packing material, 2012, isolated by Ž. Jurjević, CCF 5511 = EMSL No. 1675 = IBT 34616. **USA**, Illinois, Peoria, culture contaminant, 1975, isolated by T. G. Pridham, NRRL 25862. **USA**, New Jersey, Sicklerville, air in school auditorium, 2013, isolated by Ž. Jurjević, CCF 5512 = EMSL No. 2206 = IBT 34617. **USA**, New Jersey, green fabric covered binders, import from China, 2014, isolated by Ž. Jurjević, CCF 5513 = EMSL No. 2429. **USA**, North Carolina, mattress cover, 2012, isolated by Ž. Jurjević, CCF 5509 = EMSL No. 1611. **USA**, Illinois, O'fallon, baseball gloves in store, 2014, isolated by Ž. Jurjević, CCF 5514 = EMSL No. 2652. **USA**, cattle feed, 1984, isolated by B. W. Horn, NRRL 25882. **USA**, New Jersey, hospital air, 2012, isolated by Ž. Jurjević, CCF 5510 = EMSL No. 1633. **USA**, Alabama, home air, 2010, isolated by Ž. Jurjević, CCF 5508 = EMSL No. 1416. **USA**, New York, Upton, work station, indoor air, 2015, isolated by Ž. Jurjević, EMSL No. 2892. **USA**, California, San Diego, child carrier, 2015, isolated by Ž. Jurjević, CCF 5515 = EMSL No. 2906, EMSL No. 2746, EMSL No. 2897, EMSL No. 2748.

Aspergillus reticulatus F. Sklenar, Ž. Jurjević, S.W. Peterson & Hubka, sp. nov. MycoBank MB818940. Fig. 30.

Etymology: Referring to the lobate-reticulate ornamentation of the conidia.

Typus: **USA**, South Carolina, Charleston, lung biopsy, 1965, isolated by L. Jaynes (holotype PRM 944442, isotype PRM 944443, culture ex-type NRRL 25852 = CCF 5516 = DTO 356-D4 = IBT 34540).

Colony diam, 14 d (*mm*): M40Y 46–50; CYA 8–14; CY20S 21–30; CY20S 30 °C 8–18; CY20S 37 °C No growth; DG18 26–33; MEA 2–7; M60Y 55–60; M60Y 30 °C 50–55; M60Y 37 °C 3–20; MEA + 10 % NaCl 32–40.

Colony characters: M40Y 25 °C, 14 d: Colonies raised, surface floccose to lanose; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24E6); soluble pigment absent; exudate absent; reverse centrally golden brown (5D7) to greyish yellow (4C4), pale yellow (4A3) in margins. CYA 25 °C, 14 d: crateriform, radially wrinkled; colony surface velutinous, margin undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally greyish orange (5B3) to orange white (5A2) in margins. CY20S 25 °C, 14 d: centrally raised; colony surface velutinous to floccose, margin entire to delicately filiform; mycelial areas white; sporulation pale turquoise (24A3) to deep turquoise (24E8); soluble pigment absent; exudate absent; reverse centrally aquamarine greyish turquoise (24B3) to yellowish white (4A2) in margins. DG18 25 °C, 14 d: umbonate; colony surface floccose, margin entire to delicately filiform; mycelial areas white; sporulation aquamarine deep green (25E8); soluble pigment absent; exudate absent; reverse centrally dull green (25D3) to orange grey (5B2), yellowish white (4A2) in margins. MEA 25 °C, 14 d: cerebriform; colony surface velutinous, margin undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally greyish orange (5C5) to orange white (5A2) in margins. M60Y 25 °C, 14 d: raised; colony surface floccose to lanose; margin entire to delicately filiform; mycelial areas white; sporulation greyish green (25E6); soluble pigment absent; exudate absent; reverse centrally cinnamon brown (6D6), pale yellow (4A3) to greyish yellow (4C5), pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat to umbonate; colony surface floccose; margin entire to delicately filiform; mycelial areas white; sporulation deep turquoise (24E8); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2) to brownish orange (5C5), cream pale yellow (4A3) in margins.

Micromorphology: Conidial heads globose to radiate. Conidiophores uniseriate. Stipes smooth, densely covered by short hairs in SEM, 5–7 µm wide in the middle part, non-septate. Vesicles globose or pyriform, 14–20 µm wide. Phialides flask-shaped, densely covered by short hairs in SEM, 9–11 µm long, covering two thirds to three quarters of the vesicle. Conidia borne smooth, ellipsoidal, becoming rough-walled, globose or barrel-shaped at maturity, tuberculate to lobate-reticulate in SEM, sometimes remaining in chains, connectives evident, 3.5–4.5 × 2.5–3.5 µm.

Distinguishing characters: *Aspergillus reticulatus* is phylogenetically closely related to *A. salinicola* (Fig. 7). These two species are less xerophilic than species closely related to *A. penicillioides*, because they grow slightly faster in conditions with higher water activity and slower in conditions with lower water activity (higher concentration of salt). In contrast to *A. salinicola*, isolates of *A. reticulatus* can grow on M60Y at 37 °C, grow faster on CY20S at 25 °C and 30 °C and usually have wider stipes and larger vesicles. See also distinguishing characters of *A. penicillioides*.

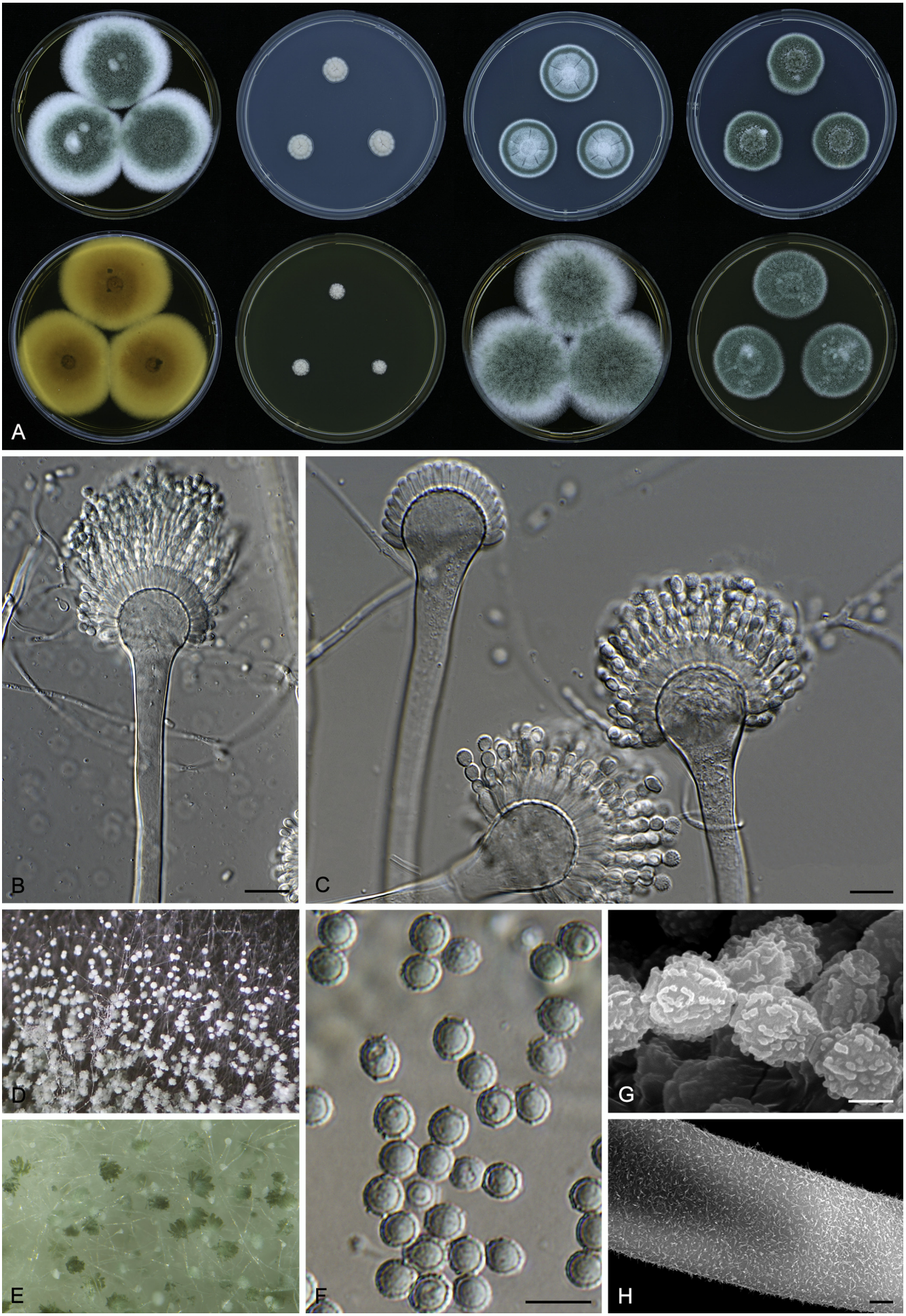


Fig. 30. *Aspergillus reticulatus*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

Additional materials examined: **Belgium**, Brussels, dust from carpet, 2008, collector unknown, IHEM 22696. **Czech Republic**, Zlín, leather shoe, 1998, isolated by T. Skálová, CCF 3112 = IBT 34634 = NRRL 62490. **France**, French madeleines, 2013, F. Déniel, UBOCC-A-115044, UBOCC-A-115045. **Puerto Rico**, Bayamon, administrative area, air, 2014, isolated by Ž. Jurjević, CCF 5522 = EMSL No. 2525, CCF 5523 = EMSL No. 2526. **Puerto Rico**, Bayamon, office air, 2014, isolated by Ž. Jurjević, CCF 5524 = EMSL No. 2548 = IBT 34637. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar and D.D. Graf, EXF-10429 = CCF 5656. **USA**, Idaho, home air, 2009, isolated by Ž. Jurjević, CCF 5518 = NRRL 58903 = EMSL No. 1272 = IBT 34819. **USA**, Idaho, home air, 2010, isolated by Ž. Jurjević, CCF 5519 = NRRL 62004 = EMSL No. 1313, CCF 5520 = NRRL 62005 = EMSL No. 1314. **USA**, Idaho, outside air, 2010, isolated by Ž. Jurjević, CCF 5521 = EMSL No. 1362 = DTO 356-E4 = IBT 34518. **USA**, Florida, home air, 2008, isolated by Ž. Jurjević, CCF 5525 = NRRL 58572 = EMSL No. 885 = IBT 34880. **USA**, Georgia, Chamblee, lung biopsy, 1950, isolated by L. Ajello, NRRL 25878 = CCF 5517. **USA**, California, San Diego, child carrier, 2015, isolated by Ž. Jurjević, EMSL No. 2778, EMSL No. 2767, EMSL No. 2750, EMSL No. 2760, EMSL No. 2770, EMSL No. 2771, EMSL No. 2772, EMSL No. 2745, EMSL No. 2756. **USA**, Hawaii, Honolulu, drawer, unknown year of isolation, isolated by J. Varga, CBS 114325 = DTO 077-H3.

Aspergillus salinicola Zalar, F. Sklenar, Visagie & Hubka, sp. nov. MycoBank MB818941. Fig. 31.

Etymology: Referring to salterns, a source of isolation of some isolates.

Typus: **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar (holotype PRM 944448, culture ex-type EXF-10401 = IBT 34266 = CCF 5526 = NRRL 66621).

Colony diam, 14 d (*mm*): M40Y 32–40; CYA (0)8–9; CY20S 9–20; CY20S 30 °C 0–5; CY20S 37 °C No growth; DG18 21–30; MEA 0–5; M60Y 42–55; M60Y 30 °C 27–43; M60Y 37 °C No growth; MEA + 10 % NaCl 32–35.

Colony characters: M40Y 25 °C, 14 d: Colonies flat to umbonate, surface velutinous; margin delicately filiform; mycelial areas pinkish white (7A2) to white; sporulation greyish green (25B5); soluble pigment absent; exudate absent; reverse centrally golden brown (5D7) to pale yellow (4A3) in margins. CYA 25 °C, 14 d: cerebriform; colony surface floccose, margin undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally greyish orange (5B3) to orange white (5A2) in margins. CY20S 25 °C, 14 d: centrally raised, radially wrinkled; colony surface velutinous, margin entire to delicately filiform; mycelial areas white; sporulation turquoise grey (24D2); soluble pigment absent; exudate absent; reverse centrally nougat dull yellow (3D4) to pale yellow (4A3) in margins. DG18 25 °C, 14 d: flat, slightly centrally raised; colony surface floccose, sometimes forming sectors, margin entire; mycelial areas white; sporulation greyish green (25D6); soluble pigment absent; exudate absent; reverse centrally greyish green (25C3) to greyish orange (5B3), orange white (5A2) in margins. MEA 25 °C, 14 d: no growth to microcolonies. M60Y 25 °C, 14 d: raised; colony surface floccose to lanose; margin filiform; mycelial areas white; sporulation greyish green (25D7); soluble pigment absent; exudate absent; reverse centrally olive green (2F6), pale yellow (4A3) to greyish yellow (4C5), pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: centrally raised; colony surface granular to filamentous, floccose in the centre; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24C4); soluble pigment absent; exudate absent; reverse centrally light brown (5D6) to amber yellow (4D6), champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads at first globose, later becoming loosely radiate. Conidiophores uniseriate. Stipes smooth, sparsely covered by short hairs in SEM, 4–6 µm wide in the middle part, non-septate. Vesicles pyriform, spatulate to subclavate, 11–14 µm wide. Phialides flask-shaped, sparsely covered by short hairs in SEM, 8–9 µm long, covering one third to one half of the vesicle. Conidia borne smooth and ellipsoidal, becoming irregularly rough-walled, globose to subglobose, tuberculate to lobate-reticulate in SEM, connectives evident, 3–4 × 2–3 µm.

Distinguishing characters: See distinguishing characters of *A. reticulatus*. A relatively unique character of *A. salinicola* is the pale colour of sporulation in comparison with other species belonging to the *A. penicillioides* clade.

Additional materials examined: **Canada**, Nova Scotia, Wolfville, house dust, 2015, collected by A. Walker, isolated by C.M. Visagie, KAS 6054. **France**, Brest, painting from Brittany (painter: J.M. Vien; painting title: Jésus et le fils du centenier de Capharnaüm; painting technique: oil on paper mounted on canvas), 2015, isolated by F. Déniel, UBOCC-A-116019 = CCF 5528 = IBT 34635. **France**, French savaroise, 2013, F. Déniel, UBOCC-A-115043 = CBS 140431 = DTO 334-E2. **Germany**, Essen, kindergarten, unknown year of isolation, isolated by J. Varga, CBS 117344 = DTO 077-I1. **Slovenia**, Sečovlje salterns, 1996, isolated by N. Gunde-Cimerman and P. Zalar, EXF-226 = CCF 5527 = IBT 34277 = NRRL 66622.

Aspergillus tardicrescens F. Sklenar, Houbraken, Zalar & Hubka, sp. nov. MycoBank MB818942. Fig. 32.

Etymology: Referring to the slow growth of the species.

Typus: **The Netherlands**, Alphen aan den Rijn, museum piece (measuring table), 2014, collector unknown (holotype PRM 944438, isotype PRM 944439, culture ex-type DTO 316-B5 = CCF 5529 = IBT 34558 = NRRL 66623).

Colony diam, 14 d (*mm*): M40Y 17–29; CYA No growth; CY20S 0–5; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 12–17; MEA No growth; M60Y 31–50; M60Y 30 °C 15–35; M60Y 37 °C No growth; MEA + 10 % NaCl 19–29.

Colony characters: M40Y 25 °C, 14 d: Colonies umbonate, surface powdery; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (water blue) (24C5); soluble pigment absent; exudate absent; reverse centrally olive brown (4F8) to greyish yellow (4C7), pale orange (5A3) in margins. CYA 25 °C, 14 d: no growth. CY20S 25 °C, 14 d: convex; colony surface velutinous, margin undulate; mycelial areas white; sporulation pale green (25A3); soluble pigment absent; exudate absent; reverse centrally greenish grey (25D2) to orange white (5A2) in margins. DG18 25 °C, 14 d: convex; colony surface floccose, margin entire; mycelial areas white; sporulation aquamarine turquoise blue (24A6); soluble pigment absent; exudate absent; reverse centrally turquoise grey (24C2) to orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: raised; colony surface floccose to cottony; margin entire to delicately filiform; mycelial areas white; sporulation aquamarine greyish turquoise (24B3) to pastel green (25A4); soluble pigment absent; exudate absent; reverse centrally brownish orange (5C5) to pale yellow (4A3) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat to umbonate; colony surface powdery to floccose; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (24C3); soluble pigment absent; exudate absent; reverse centrally bronze brown (5E5) to greyish yellow (4C7), cream pale yellow (4A3) in margins.

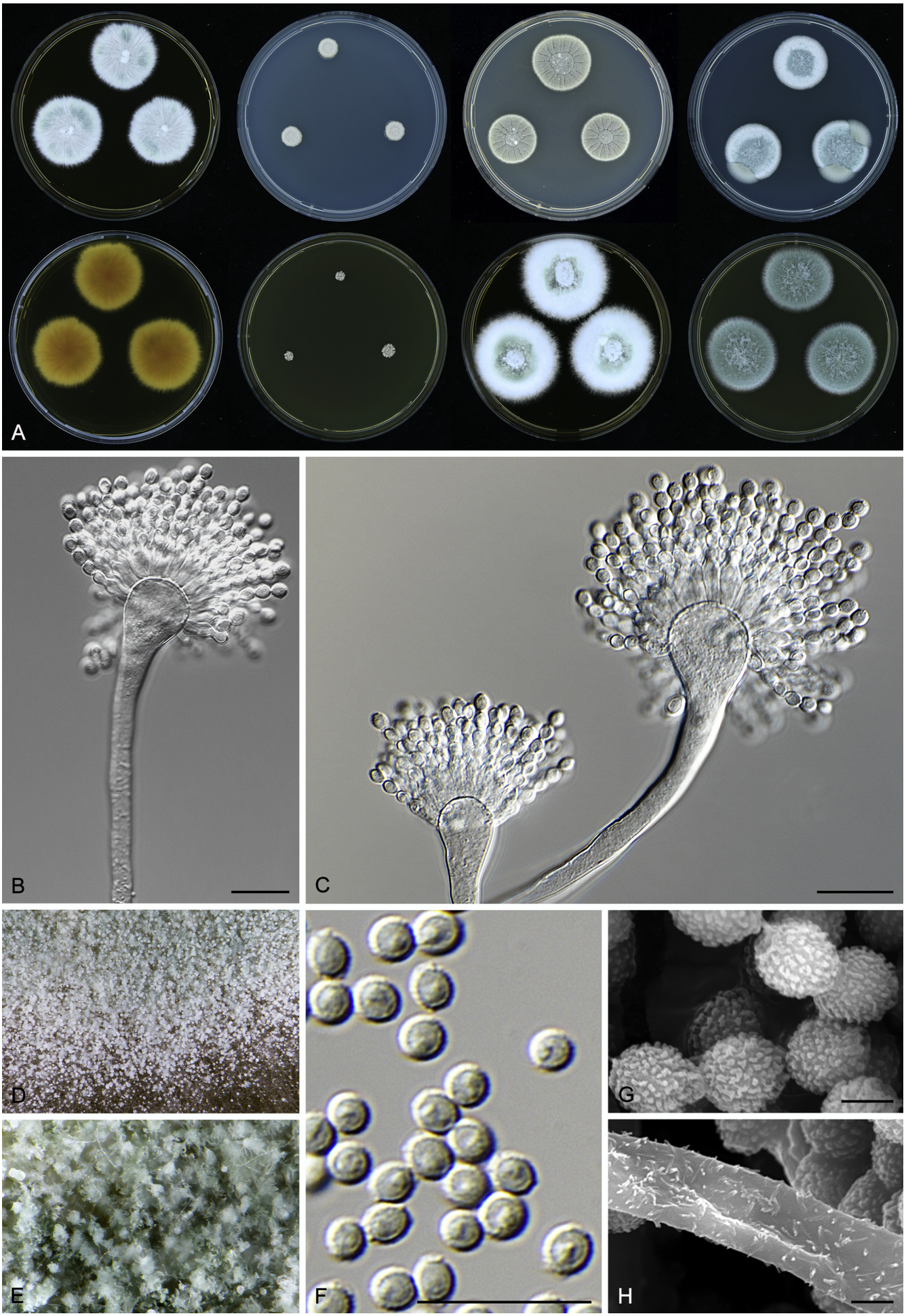


Fig. 31. *Aspergillus salinicola*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

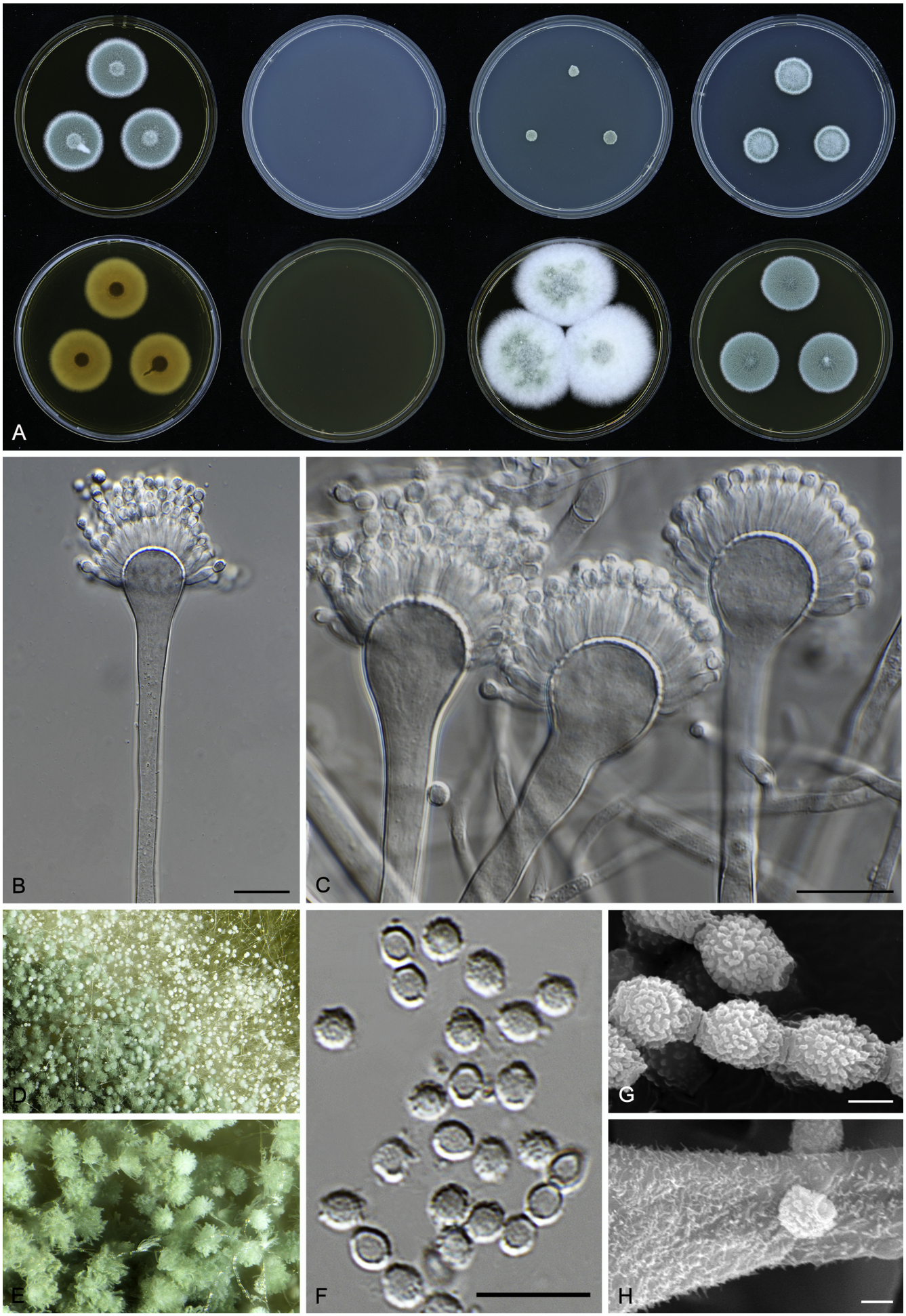


Fig. 32. *Aspergillus tardicrescens*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C.** Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

Micromorphology: Conidial heads at first globose, later becoming radiate. Conidiophores uniseriate. Stipes smooth, densely covered by short hairs in SEM, 5–8 µm wide in the middle part, with occasional septa. Vesicles subglobose, spatulate, pyriform to subclavate, 14–19 µm wide. Phialides flask-shaped, densely covered by short hairs in SEM, 8–11 µm long, covering upper half to two thirds of the vesicle. Conidia borne irregularly rough and ellipsoidal, becoming coarsely rough-walled, subglobose or barrel-shaped at maturity, tuberculate in SEM, connectives evident, 3.5–4.5 × 2.5–3.5 µm.

Distinguishing characters: See distinguishing characters of *A. penicillioides*. Conidia of *A. tardicrescens* are smaller than those of *A. clavatophorus* and *A. magnivesiculatus*; its vesicles are smaller than of *A. magnivesiculatus*. The majority of isolates is not able to grow on CY20S at 30 °C or even at 25 °C.

Additional materials examined: **Canada**, Nova Scotia, Wolfville, house dust, 2015, collected by A. Walker, isolated by C. M. Visagie, KAS 6252 = DTO 356-I5. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar and D.D. Graf, EXF-10454 = IBT 34281 = CCF 5530 = NRRL 66624. **Slovenia**, Ljubljana, air in depot of Conservation Centre of the Institute for the Protection of Cultrural Heritage of Slovenia, 2015, isolated by P. Zalar and D.D. Graf, EXF-10456 = IBT 34286. **The Netherlands**, Alphen aan den Rijn, museum piece (dentist chair), 2014, collector unknown, DTO 316-A7. **The Netherlands**, Alphen aan den Rijn, museum piece (rubber tyre of brancard), 2014, collector unknown, DTO 316-A8 = IBT 34562. **The Netherlands**, Alphen aan den Rijn, museum piece (x-ray table), 2014, collector unknown, DTO 316-A9. **The Netherlands**, Alphen aan den Rijn, museum piece (vitrine), 2014, collector unknown, DTO 316-B4. **The Netherlands**, Tilburg, moist wall of archive, 2008, isolated by J. Houbraken, DTO 073-H6. **The Netherlands**, Zwartewaal, unknown substrate, 2012, isolated by M. Meijer, DTO 231-B8. **The Netherlands**, archive material, unknown year of isolation, isolated by J. Houbraken, DTO 014-A3.

Aspergillus villosus F. Sklenar, S.W. Peterson & Hubka, sp. nov. MycoBank MB818933. Fig. 33.

Etymology: Referring to stipes and phialides, which are villose when observed using SEM.

Typus: **United Kingdom**, Scotland, Kirkhill, unknown substrate, 1945, isolated by D. Snow (holotype PRM 944430, isotype PRM 944431, culture ex-type NRRL 25813 = CCF 5531 = DTO 356-C9 = IBT 34822).

Colony diam, 14 d (*mm*): M40Y 21–23; CYA 8–10; CY20S 17–19; CY20S 30 °C 7–8; CY20S 37 °C No growth; DG18 16–20; MEA 5–6; M60Y 26–28; M60Y 30 °C 28–30; M60Y 37 °C 6–8; MEA + 10 % NaCl 22–23.

Colony characters: M40Y 25 °C, 14 d: Colonies flat, surface floccose; margin irregular, filiform; mycelial areas white; sporulation greyish green (25E7) to bluish green (25A6); soluble pigment absent; exudate absent; reverse centrally olive green (2F6) to champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: convex; colony surface floccose, margin entire to undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally yellowish grey (4B2) to yellowish white (4A2) in margins. CY20S 25 °C, 14 d: umbonate, slightly radially wrinkled; colony surface velutinous to floccose, margin entire; mycelial areas white; sporulation light turquoise (24A4) to pastel blue (23A4); soluble pigment absent; exudate absent; reverse dull green (25D3) to pale orange (5A3) in margins. DG18 25 °C, 14 d: centrally raised; colony surface floccose, margin filiform; mycelial areas pinkish white (7A2); sporulation greyish turquoise (24D5); soluble pigment absent; exudate absent; reverse centrally orange white (5A2) to yellowish white (4A2) in margins. MEA 25 °C, 14 d: convex; colony surface floccose, margin undulate; mycelial areas white; sporulation sparse; soluble pigment absent; exudate absent; reverse centrally clay brownish orange (5C5) to pale orange (5A3) in margins. M60Y 25 °C, 14 d: raised; colony surface floccose; margin irregular, filiform; mycelial areas white; sporulation pastel green (25B4); soluble pigment absent; exudate absent; reverse centrally olive grey (2F2) to champagne greyish yellow (4B4) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat; colony surface floccose, margin filiform; mycelial areas pinkish white (7A2); sporulation greyish turquoise (24D5); soluble pigment absent; exudate absent; reverse centrally cinnamon brown (6D6) to champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads loosely columnar. Conidiophores uniseriate. Stipes smooth, densely covered by long hairs in SEM, 5–6 µm wide in the middle part, widening gradually toward the vesicle, with occasional septa. Vesicles pyriform, spatulate to clavate, 10–14 µm wide. Phialides flask-shaped, densely covered by long hairs in SEM, 9–12 µm long, covering the upper half of the vesicle. Conidia borne as smooth, long cylinders, echinulate at maturity, tuberculate in SEM, ellipsoidal or barrel-shaped, connectives evident, 3.5–4.5 × 2.5–3.5 µm.

Distinguishing characters: See the distinguishing characters of *A. conicus*. The phylogenetic position of *A. villosus* in the *A. conicus* clade is not fully resolved. It is a member of the *A. conicus* clade based on the *BEAST analysis, but the concatenated dataset analysis resolves it outside the clade with absolute support. Probably its closest relatives are *A. conicus*, *A. domesticus* and *A. destruens*. *Aspergillus villosus* can be distinguished by its faster growth on CY20S, ability to grow on CY20S at 30 °C and on M60Y at 37 °C. Additionally, *A. villosus* has rather spatulate vesicles in contrast to pyriform or ellipsoidal vesicles in mentioned relatives; conidia ornamentation in SEM is tuberculate, rather than aculeate common in the *A. conicus* clade.

Additional materials examined: **France**, Brest, painting from Brittany (painter: A.D. Hondius; painting title: Jésus à la piscine de Bethsaïda ou La guérison du paralytique; painting technique: oil on canvas), 2015, isolated by F. Déniel, UBOCC-A-116020 = CCF 5532 = IBT 34624.

Aspergillus vitricola Ohtsuki, Bot. Mag. (Tokyo) 75: 436. 1962, emended description. MycoBank MB326665. Fig. 34.

Typus: **Japan**, binocular lens, 1962, collector unknown (holotype in the Herbarium of the Nagao Institute, culture ex-type NRRL 5125 = DTO 356-F7 = ATCC 16905 = ATCC 36505 = IMI 108298 = CCF 5533 = IBT 34530).

Colony diam, 14 d (*mm*): M40Y 19–36; CYA No growth; CY20S (0)4–7; CY20S 30 °C No growth; CY20S 37 °C No growth; DG18 8–18; MEA No growth; M60Y 25–42; M60Y 30 °C 16–42; M60Y 37 °C No growth; MEA + 10 % NaCl 9–26.

Colony characters: M40Y 25 °C, 14 d: Colonies flat, wrinkled, surface velutinous; margin entire to delicately filiform; mycelial areas white; sporulation greyish turquoise (water blue) (24C5); soluble pigment absent; exudate absent; reverse centrally olive green (2F6) to olive yellow (3D6), champagne greyish yellow (4B4) in margins. CYA 25 °C, 14 d: no growth. CY20S 25 °C, 14 d: no growth to microcolonies. DG18 25 °C, 14 d: centrally raised; colony surface velutinous, margin entire to delicately filiform; mycelial areas white; sporulation sparse, turquoise white (24A2); soluble

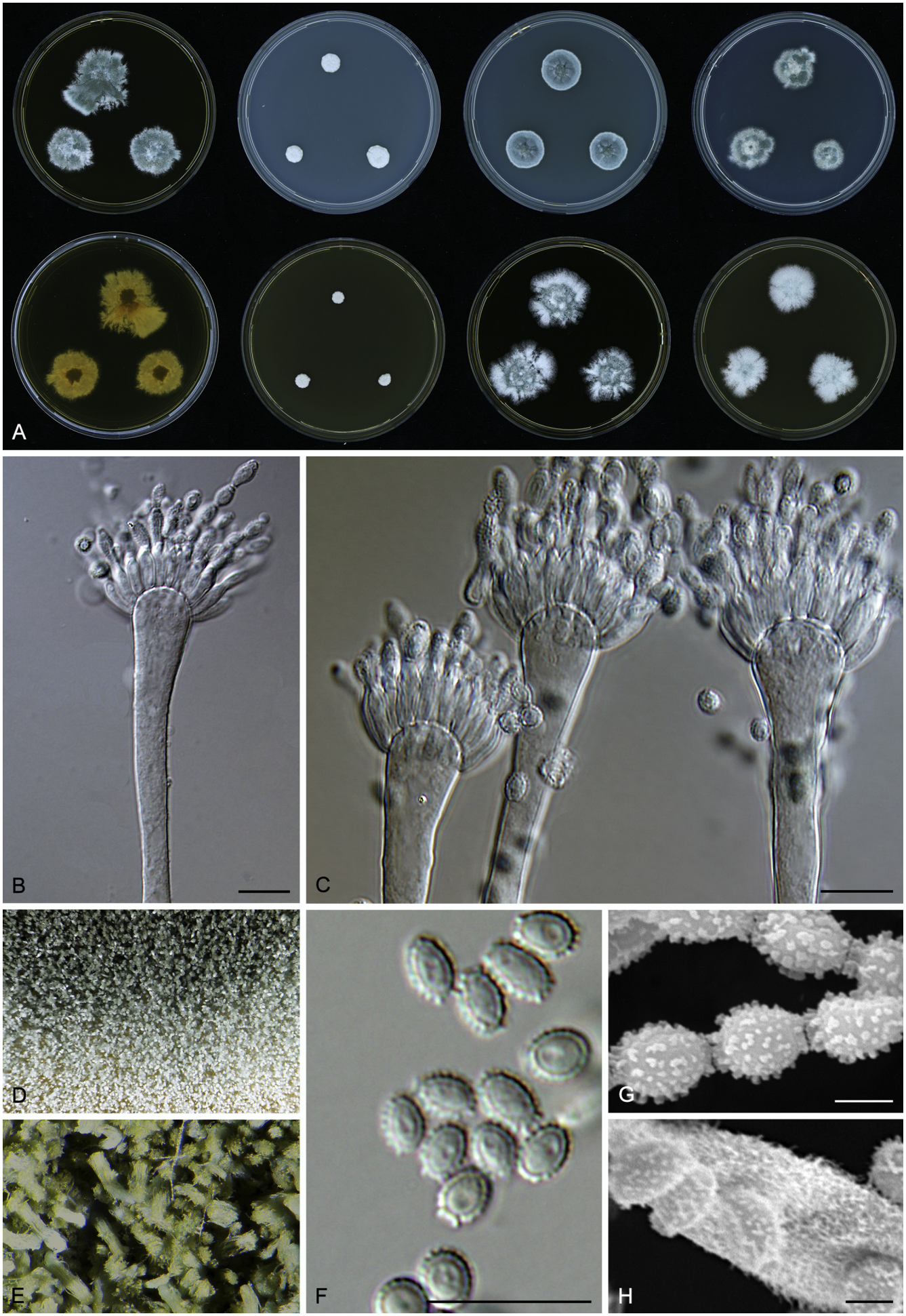


Fig. 33. *Aspergillus villosus*. **A**. Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C**. Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

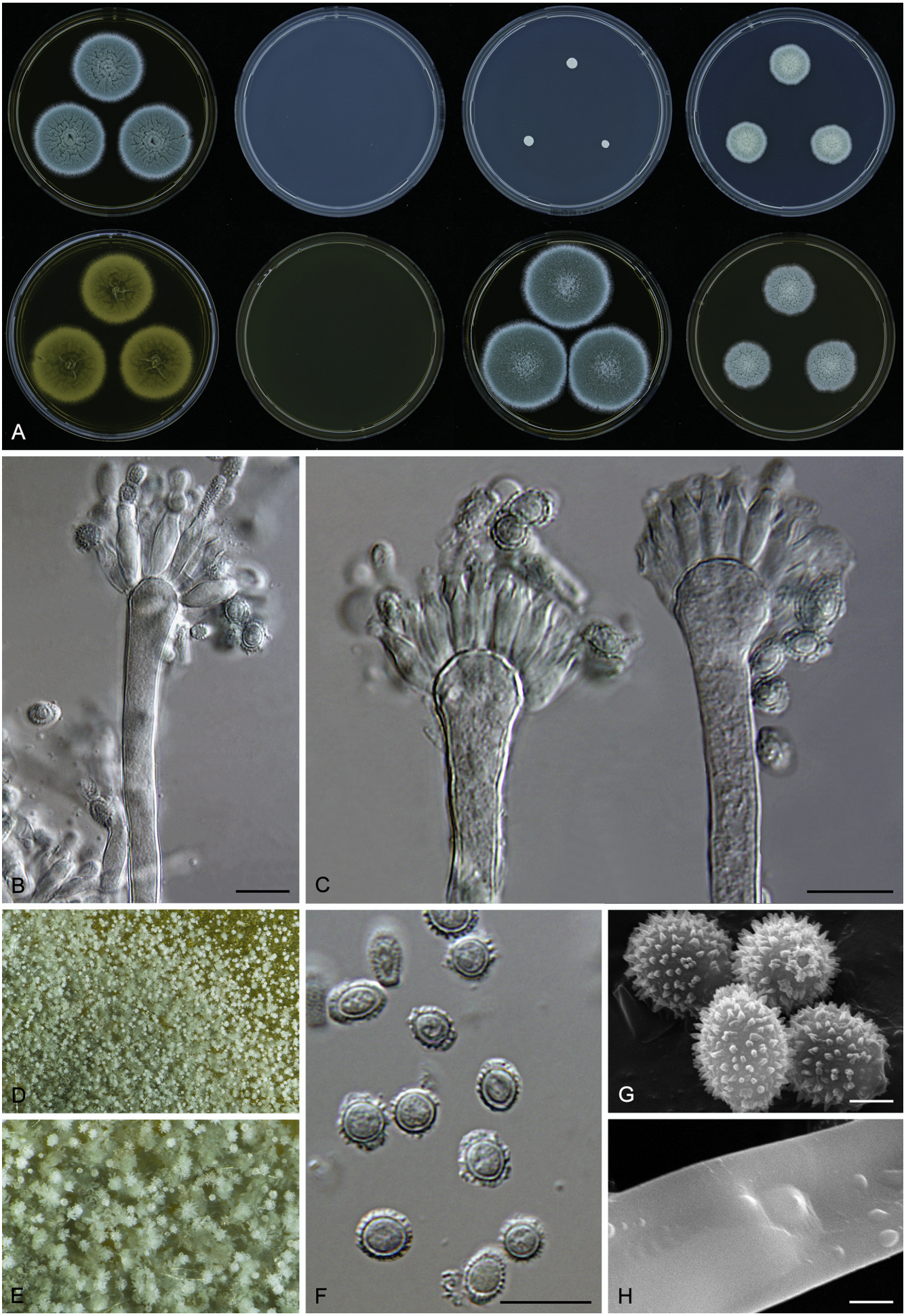


Fig. 34. *Aspergillus vitricola*. **A.** Colonies after 14 d at 25 °C: top row left to right, obverse M40Y, CYA, CY20S and DG18; bottom row left to right, reverse M40Y, MEA, M60Y and MEA + 10 % NaCl. **B, C**. Conidiophores. **D, E.** Conidial heads. **F.** Conidia. **G.** Conidia in SEM. **H.** Stipe in SEM. Scale bars: B, C, F = 10 µm; G, H = 2 µm.

pigment absent; exudate absent; reverse centrally turquoise white (24D2) to orange white (5A2) in margins. MEA 25 °C, 14 d: no growth. M60Y 25 °C, 14 d: flat to slightly centrally raised; colony surface velutinous to powdery; margin entire to delicately filiform; mycelial areas white; sporulation deep turquoise (25E8); soluble pigment absent; exudate absent; reverse centrally dark green (29F3) to champagne greyish yellow (4B4) in margins. MEA + 10 % NaCl 25 °C, 14 d: flat, wrinkled; colony surface velutinous, margin entire to delicately filiform; mycelial areas white; sporulation aquamarine greyish turquoise (24B3); soluble pigment absent; exudate absent; reverse centrally cinnamon brown (6D6) to olive yellow (3D6), champagne greyish yellow (4B4) in margins.

Micromorphology: Conidial heads radiate. Conidiophores uniseriate. Stipes smooth in SEM, 4.5–6 µm wide in the middle part, widening gradually toward the vesicle, with occasional septa. Vesicles spatulate, pyriform to clavate, 7–12 µm wide. Phialides flask-shaped, 8–10 µm long, covering the upper half to two thirds of the vesicle. Conidia borne as rough-walled long cylinders, echinulate at maturity, aculeate in SEM, subglobose to ellipsoidal, connectives evident, commonly remaining in chains, 4.5–5.5 × 3–4 µm.

Distinguishing characters: Isolates of *A. vitricola* have larger conidia, narrower stipes and much smaller vesicles compared to *A. glabripes*. *Aspergillus vitricola* does not grow on MEA and CYA at 25 °C and CY20S at 30 °C and exhibit only very restricted growth on CY20S at 25 °C. Both species are also different micromorphologically and in growth parameters on most media.

Additional materials examined: **Canada**, British Columbia, Victoria, house dust, 2015, collected by B. Kendrick, isolated by C.M. Visagie, KAS 6093, KAS 6087 = DTO 356-H2 = IBT 34532, KAS 6199, KAS 6237 = DTO 356-I2, KAS 6238, KAS 6281. **Canada**, New Brunswick, Little Lepreau, house dust, 2015, collected by A. Walker, isolated by C.M. Visagie, DAOMC 251500 = KAS 6133, KAS 6086, KAS 6178 = DTO 356-H8, KAS 6279. **Canada**, Nova Scotia, Wolfville, house dust, 2015, collected by A. Walker, isolated by C.M. Visagie, KAS 6150 = DTO 356-H5 = IBT 34531, KAS 6235, KAS 6236, KAS 6250, KAS 6251, KAS 6263, KAS 6264, KAS 6278 = DTO 356-I8, KAS 6285, KAS 6287, KAS 6288, KAS 6290. **Canada**, Ontario, Stittsville, house dust, collected by K.A. Seifert, isolated by C.M. Visagie, KAS 5687. **Slovenia**, Ljubljana, mouldy hat of a national costume of a folklore group (stored in basement depot), 2015, isolated by P. Zalar, EXF-10301 = CCF 5654 = IBT 34290. **Slovenia**, Ljubljana, oil painting on canvas, 2015, isolated by P. Zalar and D.D. Graf, EXF-10383 = CCF 5655 = IBT 34272. **Slovenia**, Ljubljana, oil painting on canvas, 2012, isolated by P. Zalar, EXF-7700 = CCF 5657 = IBT 34282 = IBT 33575. France, raisin madeleines, 2014, isolated by F. Déniel, CBS 140435 = UBOCC-A-115047 = DTO 334-E6. **The Netherlands**, archive material, 2006, isolated by J. Houbraken, DTO 014-A4. **The Netherlands**, Noordwijk, carpet "zeegrastapijt" in house, 2015, J. Houbraken, DTO 331-D6. **The Netherlands**, Gorinchem, archive material, 2010, isolated by M. Meijer and O. Terhoeven, DTO 122-I4, DTO 123-B2, DTO 122-I5. **USA**, California, San Diego, child carrier, 2015, isolated by Ž. Jurjević, CCF 5534 = EMSL No. 2785.

Dichotomous key to species from section *Restricti*

1a) Homothallic, no growth on M40Y at 25 °C ..... *A. halophilicus*
1b) Anamorphic, good growth on M40Y at 25 °C 2

2a) Conidial heads from colony edge radiate or globose when young 11
2b) Conidial heads columnar or loosely columnar when young 3

Aspergillus restrictus/*conicus* clades

3a) No growth on CY20S at 30 °C and M60Y at 37 °C after 14 d 4
3b) Growth on CY20S at 30 °C and M60Y at 37 °C after 14 d 8

4a) Colonies on M60Y at 25 and 30 °C exceed 40 mm after 14 d 5
4b) Colonies on M60Y at 25 and 30 °C do not exceed 40 mm after 14 d 6

5a) Stipes 3–5 µm wide, vesicles 6.5–9 µm wide, phialides 7.5–9 µm long, reverse colour on M40Y olive grey in the centre ..... *A. gracilis*
5b) Stipes 4–6 µm wide, vesicles 9–13 µm wide, phialides 9–10.5 µm long, reverse colour on M40Y cinnamon brown in the centre ..... *A. pseudogracilis*

6a) Sporulation colour on M40Y myrtle green, reverse apricot greyish orange in the centre ..... *A. destruens*
6b) Sporulation colour on M40Y turquoise, reverse olive grey in the centre 7

7a) Sporulation colour on M40Y pale turquoise, conidia predominantly globose to subglobose (or barrel-shaped) ..... *A. conicus*
7b) Sporulation colour on M40Y greyish turquoise, conidia predominantly ellipsoidal (or barrel-shaped) ..... *A. domesticus*

8a) Length of phialides ≥ 9 µm, colonies on CY20S at 30 °C and CYA at 25 °C and M60Y at 37 °C ≤ 10 mm after 14 d, conidia tuberculate in SEM ..... *A. villosus*
8b) Length of phialides ≤ 9 µm, colonies on CY20S at 30 °C and CYA at 25 °C and M60Y at 37 °C > 10 mm after 14 d, conidia aculeate in SEM 9

9a) Diameter of vesicle ≥ 16 µm ..... *A. pachycaulis*
9a) Diameter of vesicle ≤ 16 µm 10

10a) Conidia 3.5–4.5 × 2.5–3 µm, vesicle diameter 9–12 µm ..... *A. restrictus*
10b) Conidia 4.5–6 × 3–4.5 µm, vesicle diameter 11–16 µm ..... *A. caesiellus*

Aspergillus penicillioides/*vitricola* clades

11a) Average diameter of vesicles ≥ 19 µm 12
11b) Average diameter of vesicles < 17 µm 13

12a) Conidia 3.5–4.5 × 2–3 µm, length of phialides 7–9 µm ..... *A. glabripes*
12b) Conidia 4.5–5.5 × 3.5–4.5 µm, length of phialides 9–11 µm ..... *A. magnivesiculatus*

13a) Length of phialides ≤ 8 µm, average length ~ 7 µm ..... *A. canadensis*
13b) Length of phialides ≥ 8 µm, average length ≥ 8.5 µm 14

14a) Conidia do not exceed 4 µm in long axis, average length ca 3.6 µm ..... *A. salinicola*
14b) Conidia commonly exceed 4 µm in long axis, average length ca 4–5 µm 15

Table 10. Exometabolites reported from *Aspergillus* section *Restricti* isolates.

Exometabolite	Producer	Reference
Arestrictin A and B	*A. restrictus*	Itabashi *et al.* (2006)
Arestrictin A and B	*A. penicillioides*	Itabashi *et al.* (2006)
Cristatin A	*A. restrictus*	Itabashi *et al.* (2006)
Neoechinulin A	*A. halophilicus*	Micheluz *et al.* (2016)
Asperglaucide (= aurantiamide acetate)	*A. penicillioides*	Isshiki *et al.* (2001); Micheluz *et al.* (2016)
Asperglaucide (= aurantiamide acetate)	*A. restrictus*	Micheluz *et al.* (2016)
Rugulusovin	*A. halophilicus*	Micheluz *et al.* (2016)
Citreorosein	*A. penicillioides*	Micheluz *et al.* (2016)
Deoxybrevianamide A	*A. penicillioides*	Micheluz *et al.* (2016)
Deoxybrevianamide E	*A. halophilicus*	Micheluz *et al.* (2016)
Exometabolites incorrectly ascribed to sect. *Restricti* species		
Restrictocin	*A. restrictus*[1]	Lamy and Davies (1991), Arruda *et al.* (1992), Brandhorst & Kenealy (1992), Yang & Kenealy (1992), Brandhorst *et al.* (1996)
Chaetoviridin A	*A. halophilicus*	Micheluz *et al.* (2016)
Pseurotin A & D	*A. halophilicus*[2]	Micheluz *et al.* (2016)
Tenellin	*A. halophilicus*[2]	Micheluz *et al.* (2016)
Tryprostatin B	*A. halophilicus*[2]	Micheluz *et al.* (2016)

[1] The isolates producing restrictocin have been re-identified as *A. fumigatus* (strains MDH 13462L = ATCC 34475 = NRRL 2869 and MDH 17070 = NRRL 3050 = ATCC 34506) (Arruda *et al.* 1992); see also Jong *et al.* (1996).
[2] One of three isolates identified as *A. halophilicus* produced metabolites that are present in *A. fumigatus* (Frisvad *et al.* 2009, Frisvad & Larsen 2016), so it was probably contaminated with *A. fumigatus*.

15a) Colony diameter on CY20S and M40Y at 25 °C after 14 d > 20 and 45 mm, respectively …… *A. reticulatus*
15b) Colony diameter smaller on both media …….. 16

16a) Average width of stipes ≤ 5.5 µm ……17
16b) Average width of stipes frequently ≥ 6 µm ……..… 19

17a) No production of soluble pigment on DG18, average length of conidia > 4.5 µm, stipes smooth in SEM …….. *A. vitricola*
17b) Greyish yellow soluble pigment on DG18, average length of conidia ca 4 µm, stipes covered by hairs in SEM …….. 18

18a) Sporulation colour on M40Y deep green, conidia in SEM tuberculate …….. *A. penicillioides*
18b) Sporulation colour on M40Y dark turquoise, conidia in SEM lobate-reticulate …….. *A. hordei*

19a) No growth on CYA at 25 °C after 14 d, colony reverse on M40Y in shades of yellow or brown, conidia tuberculate in SEM …….. 20
19b) Growth on CYA at 25 °C after 14 d, colony reverse on M40Y dark green, conidia aculeate in SEM …….. *A. infrequens*

20a) Sporulation colour on M40Y greyish green, average dimensions of conidia 4.8 × 3.5 µm, colony diameter on CY20S at 25 °C after 14 d > 6 mm ….. *A. clavatophorus*
20b) Sporulation colour on M40Y greyish turquoise (water blue), average dimensions of conidia 4 × 2.9 µm, colony diameter on CY20S at 25 °C after 14 d < 6 mm …….. *A. tardicrescens*

DISCUSSION

Past and current concept of section *Restricti*

Thom & Church (1926) were the first who studied the classification of the species of sect. *Restricti*. They placed *A. penicilliodies*, *A. gracilis* and *A. conicus* into a transitional group between *A. glaucus* and *A. fumigatus*. Thom & Raper (1945) included also *A. restrictus* and considered the members of sect. *Restricti* as a series within the *A. glaucus* group. The *A. restrictus* group was subsequently introduced by Raper & Fennell (1965) who added also *A. caesiellus* and treated *A. vitricola* as a synonym of *A. penicillioides*. Although the first described species was *A. penicillioides*, the authors decided to name the group after the species (*A. restrictus*) they considered most important. The authors also observed variability within *A. restrictus* and mentioned unique growth characterictics of isolate NRRL 145. This isolate is in the present study designated as the ex-type of a novel species *A. destruens*, a member of the *A. conicus* clade. This species resembles *A. conicus* rather than *A. restrictus* in almost all phenotypic characters.

The taxonomic rank of section was later introduced by Samson & Gams (1985). The number of species forming the

section was subsequently reduced to three by Pitt & Samson (1990), rejecting *A. conicus* and *A. gracilis*. Even though the morphological variability within the section is rather small, at least the clades (*A. restrictus*, *A. conicus*, *A. vitricola* and *A. penicillioides*) can be clearly distinguished solely based on morphology.

Molecular studies conducted by Peterson (2000) confirmed that re-expansion of the section was necessary, supported recognition of at least six anamorphic species and placed *A. halophilicus*, which produces a homothallic teleomorph similar to those seen in sect. *Aspergillus*, into the sect. *Restricti*.

In this study, we performed a new revision of sect. *Restricti* that involved a large number of isolates and used both phenotypic and molecular genetic data for species delimitation. The number of accepted species was tripled compared to previous studies and 14 new species were described. The genetic distances between the species are greater (similarity between sequences of sister species within the clades varies from 95 % to 97 %, sequence similarity between the clades is only slightly above 90 %) than typically seen in other sections of the genus *Aspergillus*, including sister sect. *Aspergillus*. Our study could not sufficiently cover all common isolation sources of sect. *Restricti* members and description of new taxa in future can be expected if studies with extensive sampling and focused on particular substrates or environments are conducted. This assumption is supported by a comparison of our generated ITS barcodes with 1061 sect. *Restricti* 454-pyrosequences from Amend *et al.* (2010). A phylogenetic tree (Fig. 8) confirms that there is still uncovered species diversity within section *Restricti* and discovery of new species is expected in future. The data showed that the house dust conceals high number of species that belong to all species complexes recognized in this study including *A. halophilicus*. *Aspergillus penicillioides*, *A. vitricola*, *A. hordei*, *A. tardicrescens* and *A. magnivesiculatus* belonged to the most abundantly detected species. Several undescribed phylogenetic lineages are present in *A. restrictus*/*conicus* clade and one unnamed lineage sister to *A. tardicrescens* is present in *A. penicillioides* clade (Fig. 8).

Two of the 21 species accepted in this work were represented only by one isolate and three species by only two isolates. This situation, although not often discussed, is very common in the taxonomy of fungi (Lim *et al.* 2012). Even though working with species with no infraspecific variability is problematic, it is a general practice to include also these species into the analyses because the authors always want to cover all known variability (Ahrens *et al.* 2016). The results of some phylogenetic methods may be biased due to the presence of species with low or no variability, and population genetic methods cannot be used at all with datasets containing species without infraspecific variability. Since the aim of taxonomic revisions and monographs is to describe all current knowledge we included all species irrespective of the number of isolates and presence or absence of infraspecific variability. When we re-inspect the results of our phylogenetic analyses, it is common that the unresolved nodes are occupied by those species represented by a few isolates. Better resolution of phylogenetic relationships can be expected when the inevitable additional strains of rare species and discovery of novel taxa are made.

Phylogeny of subgenus *Aspergillus*

In contrast to sect. *Restricti*, sect. *Aspergillus* forms a compact clade. The phylogenetic analysis across species in subg. *Aspergillus* strongly supported the monophyly of both, sect. *Restricti* and sect. *Aspergillus* (Figs 1, 2). This topology is in agreement with trees published by Peterson (2008) and Peterson *et al.* (2008), however, especially in the latter study the sect. *Restricti* received only limited statistical support. In the more recent studies of Houbraken & Samson (2011), Houbraken *et al.* (2014) and Kocsubé *et al.* (2016), members of section *Restricti* did not form a separate monophyletic clade with respect to sister sect. *Aspergillus*. This was probably caused by the inadequate taxon sampling (low number of species included). Large genetic distances between particular clades within sect. *Restricti* could warrant proposal of several new sections (e.g., *A. restrictus*+*A. conicus*+*A. vitricola* clades = section *Restricti* s. str.; *A. penicillioides* clade; and *A. halophilicus*) solely based on phylogenetic distances (Figs 1, 2) and their comparison with those between sections in other subgenera. Several factors prevent us doing so. Most importantly, the section forms a coherent group in terms of morphology (except *A. halophilicus*) and is strikingly different from homothallic species of sister sect. *Aspergillus* that produce abundant ascomata in vitro. When viewing the phylogenetic tree of the subgenus in the radial form (Fig. 2), the real relationships between sections and clades seem clearer. There are at least three clades in sect. *Restricti* separated by large genetic distances that are similar to distances of these clades from basal taxa of sect. *Aspergillus*. One could even argue that there are enough clades on the tree spread along the transition between sections *Restricti* and *Aspergillus* to merge both sections into one (Fig. 2). Another problem is the unresolved position of some taxa belonging to sect. *Restricti*, especially *A. halophilicus*. Considering the uniqueness of *A. halophilicus* in terms of morphology, physiology, reproductive strategy in addition to phylogenetic isolation, it might seem appropriate to exclude this species from sect. *Restricti* and create a separate, single-species section. However, such action would probably result in a paraphyletic sect. *Restricti* due to the unclear position of *A. halophilicus*. Isolation of additional taxa related to *A. halophilicus* could improve results of the phylogenetic analyses and provide new phenotypic data supporting or contradicting this scenario. Furthermore, the sexual reproductive strategy of anamorphic species from sect. *Restricti* is unknown but discovery of a sexual state would not be unexpected in light of recent developments in this field (Horn *et al.* 2009, O'Gorman *et al.* 2009, Horn *et al.* 2011, Nováková *et al.* 2014).

Exometabolites

All species in *Aspergillus* sect. *Restricti* produced asperglaucide, asperphenamate or both. This is in contrast to species of *Aspergillus* sect. *Aspergillus* that do not produce these compounds (Chen *et al.* 2017). On the other hand the species in both sections do produce some extrolites in common. Mycophenolic acid is produced by several species from both sections, similarly to echinulin and derivatives such as neoechinulin-type metabolites (Chen *et al.* 2017). In sect. *Restricti* no isolates were found to produce auroglaucins or epiheveadride, compounds which are very common in the ascomata of *Aspergillus* sect. *Aspergillus*. The ascomata of *A. halophilicus* do not contain the yellow auroglaucin or flavoglaucin compounds. It is interesting to note that antarone A and asperentins produced by *A. salinicola* are

Fig. 35. Different kinds of food and commodities on which members of section *Restricti* are to be found. **A–F.** Various bakery products: **A.** Chocolate chip cookies ("*A. penicillioides*" by morphology – isolate not included in this study; isolated together with *A. cibarius*). **B.** Mouldy bread. **C.** Madeleines (isolate UBOCC-A-115045 – *A. reticulatus*). **D.** French savaroise (isolate UBOCC-A-115043 – *A. salinicola*). **E.** French mini cakes (isolate UBOCC-A-115048 – *A. domesticus*). **F.** Mouldy cake (mixed spoilage with *Penicillium* and *Eurotium* spp.). **G.** Grain containing diverse fungal populations similar to nuts, spices and seeds. **H.** Cigars ("*A. penicillioides*" by morphology – isolate not included in this study).

shared with a halotolerant *Penicillium antarcticum* (Vansteelandt *et al.* 2012, Geiger *et al.* 2013).

The asperglaucides and asperphenamate are simple phenylalanine derived compounds with anticancer activity (Bladt *et al.* 2013). These compounds have been found in many plants (Frisvad *et al.* 2013), and one can speculate that these medicinal compounds are not produced by the plants but rather by its endophytic fungi, including possibly endophytic isolates of *Aspergillus* sect. *Restricti*.

A very high chemical diversity was observed in section *Restricti*, but a large number of extrolites have until now only been found in this section. The chemoconsistency on the species

Fig. 36. Overview of some substrates and habitats associated with members of section *Restricti*. **A.** Solar salterns at the Adriatic coast, Sečovlje, Slovenia (isolate EXF 226 – *A. salinicola*). **B.** Indoor environment, a source of numerous species: one of the sampling sites in Canada where house dust was collected. **C.** Water damaged wall surface, dry at the time of sampling (isolate DTO 073-H6 – *A. tardicrescens*). **D.** Mouldy chair backrest (isolate DTO 231-B9 – *A. domesticus*). **E.** Textiles ("*A. penicillioides*" by morphology – isolate not included in this study). **F.** Textile water hose imported from China ("*A. penicillioides*" and *Cladosporium* sp. by morphology – isolates not included in this study). **G.** Leather baseball gloves (isolate CCF 5500 – *A. penicillioides* and CCF 5514 – *A. restrictus*). **H.** Leather armrest of a dentist chair (isolate DTO 316-A7 – *A. tardicrescens*). **I.** Skeleton of *Elephas maximus* (Asian elephant) ("*A. penicillioides*" by morphology – isolate not included in this study). **J.** Mouldy sclerotium of *Corallocytostroma ornicopreoides* imported from Australia (isolate CCF 3364 – *A. restrictus*).

level appeared to be less pronounced than in for example section *Aspergillus*, but the absence of metabolites may be caused by poor growth in some cases. For example *A. villosus* NRRL 25813^{T}, IBT 22529, IBT 22530 and IBT 22546 produced a large number of orthosporins, that were not detected in the strain UBOCC-A-116020. In such cases other media than those used here may be more suitable for production of a broader spectrum of metabolites. Addition of a high concentration of salts or sugars strongly increased the growth rate of isolates from section *Restricti*, and also secondary metabolite production. More media, such as potato dextrose agar and malt agars may be more suitable for secondary metabolite production if they are supplemented with more salt or sugar. The overview of metabolites produced by species from section *Restricti* reported from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B
C
D
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previous studies is given in Table 10. Most secondary metabolites reported from *A. halophilicus* by Micheluz *et al.* (2016), such as chaetoviridin A, deoxybrevianamide E, pseurotin A and D, rugulosuvine, stachybotryamide, tenellin and tryprostatin B could not be recovered from any strain of *A. halophilicus* examined in this study. However these extrolites may have been detected because of contamination of the YES 15 % medium (YES agar with 15 % NaCl) they used. Several extrolites detected by Micheluz *et al.* (2016) are products of *Aspergillus fumigatus*, including pseurotins A, D, tenellin and tryprostatin (Frisvad and Larsen, 2016), and were only detected in one of three examined isolates. However, YES agar with 15 % NaCl has not been used in this study, so a final conclusion on the production of these *A. fumigatus* metabolites by *A. halophilicus* must await further testing.

Ecology and pathogenicity

Species of sect. *Restricti* occur worldwide and they used to be most frequently isolated from food, feed and indoor environments. Pitt & Hocking (2009) summarised spoilage of different foods (e.g., flour, wheat, maize, rice, dried, cured or salted meat, dried fruit, spices, beans, nuts, coffee beans and salted dried fish) by *A. restrictus* and *A. penicillioides*. *Aspergillus penicillioides* is probably the most frequently isolated species from the section although it may be partly due to the broad concept adopted in the past. Members of sect. *Restricti* are commonly isolated from grain, such as corn, wheat and barley, and many species were found on grain during this study, namely *A. penicillioides*, *A. restrictus*, *A. caesiellus*, *A. magnivesiculatus*, *A. hordei*, *A. halophilicus*, *A. infrequens*. The isolates from food examined in this study originated mostly from candy and bakery products (*A. penicillioides*, *A. restrictus*, *A. glabripes*, *A. destruens*, *A. domesticus*, *A. conicus*, *A. vitricola*, *A. salinicola*), rarely from other foods such as cheese (*A. destruens*), nuts (*A. penicillioides*, *A. conicus*), tea (*A. clavatophorus*), katsuobushi (*A. magnivesiculatus*), etc. Some examples of spoiled foods examined in this study are shown in Fig. 35. The species are also commonly isolated from leather and textile goods, e.g. shoes, baseball gloves (see Fig. 36 G, H) or mattress covers (*A. penicillioides*, *A. restrictus*, *A. reticulatus*, *A. caesiellus*). The presence of these species in the indoor environment and house dust is well-known from past studies (Samson & Lustgraaf 1978, Frisvad & Gravesen 1994, Meklin *et al.* 2004, Kaarakainen *et al.* 2009, Amend *et al.* 2010, Visagie *et al.* 2014). Eleven species which were isolated from indoor air are included in our study, namely *A. caesiellus*, *A. conicus*, *A. destruens*, *A. domesticus*, *A. glabripes*, *A. magnivesiculatus*, *A. pachycaulis*, *A. penicillioides*, *A. restrictus*, *A. reticulatus* and *A. tardicrescens*. Examples of sect. *Restricti* members growing in various conditions, including indoor environment and textile can be found in Fig. 36. In a world-wide survey of fungi occurring in house dust using a portion of the ID locus as used here (Amend *et al.* 2010), *A. penicillioides* was a common OTU identified. In these analyses a 97 % cutoff is most commonly used, however, the low amount of sequence data available for sect. *Restricti* before this study makes the analysis of species diversity complicated. This is clear from our sect. *Restricti* phylogeny (Fig. 8) incorporating newly generated ITS barcodes and 454-pyrosequences from Amend *et al.* (2010), where we can now positively identify 14 of the OTU's down to species level, in contrast to the at most seven previously possible in theory. One of the main problems is the high sequence divergence between sect. *Restricti* species where culture independent methods had almost no chance of getting to a correct species name. In this study, we release more than 180 ITS rDNA barcode sequences covering both infra- and interspecies variation. This will greatly aid these modern techniques to identify common indoor fungi. A significant portion of strains and new species examined in this study was isolated from air, especially from indoor air of various regions in the USA. It seems that this enviroment might still be an important source of undiscovered genetic variability and new species. Many specimens examined in this work also come from house dust; for instance isolates of *A. canadensis* thus far originate exclusively from house dust in Canada.

The species can also cause significant damage to specimens deposited in herbaria (Zhang *et al.* 2016) and also to works of art, especially paintings (Fig. 37). Examination of fungi isolated from deteriorated paintings from Musée des beaux Arts de Brest (France) and Institute for the Protection of Cultural Heritage of Slovenia during this study showed that species *A. destruens*, *A. conicus*, *A. domesticus*, *A. vilosus*, *A. vitricola*, *A. glabripes*, *A. tardicrescens*, *A. reticulatus*, *A. magnivesiculatus* and *A. salinicola* can deteriorate paintings and generate cultural and economic losses. *Aspergillus clavatophorus* was also isolated from a painting in the Netherlands, while *A. halophilicus* was recently identified as a causative agent of book biodeterioration in archives and libraries from Micheluz *et al.* (2015).

Hypersaline water has proved to be a rich source of xerophilic fungi (Gunde-Cimerman *et al.* 2000), including members of sect. *Restricti* (Cantrell *et al.* 2006, Nazareth & Gonsalves 2014). The novel species *A. salinicola* described in this study is a saltern inhabitant (Fig. 36. A).

Some species can be isolated from very uncommon substrates as in the case of *A. vitricola* that was isolated from glass in Japan and is able to damage optical lenses (Ohtsuki 1962). As we found here, this species is also very common in house dust in Canada.

The pathogenic potential of sect. *Restricti* seems to be low considering the small number of reported cases of opportunistic human infections. Several records of human infection caused by *A. penicillioides* and *A. restrictus* is summarised by de Hoog *et al.* (2009), but the identification of the pathogens was performed exclusively by morphological examination. It is very likely that some records represent confusion with *A. fumigatus* due to similar sporulation colour and micromorphology. However, a confirmed case of invasive aspergillosis in human has been described recently that was probably caused by an undescribed species from the *A. penicillioides* clade (ITS GenBank: KP316198) (Gupta *et al.* 2016). This case demonstrated that athough the species of the sect. *Restricti* are rarely found as pathogens, they have at least limited pathogenic potential.

Fig. 37. Deteriorated paintings and books from archives. **A–H.** Deteriorated painting from Capuchin monastery of St. Francis, Ajdovščina, Slovenia (**A**) and Musée des beaux Arts in Brest, France (**B–H**); isolated species: *A. destruens, A. domesticus, A. conicus, A. glabripes, A. magnivesiculatus, A. reticulatus, A. salinicola, A. tardicrescens, A. villosus, A. vitricola.* **I–M**. Deteriorated books from archives; isolated species: *A. vitricola* and *A. domesticus*.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.simyco.2017.09.002.

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